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Application of fuzzy cluster analysis to Lake Simcoe crustacean zooplankton community structure

Kenneth H. Nicholls and Claudiu Tudorancea

Abstract: Fuzzy clustering generates cluster membership weights that indicate how tightly each object is linked to its cluster relative to other clusters of a dendrogram. In a fuzzy clustering of the crustacean-zooplankton taxa of Lake Simcoe, a large (720 km^2) hardwater lake in Ontario, Canada, we show how the membership weights can be used to rank all taxa for their contribution to the sampling unit (SU) classification, where the total number of SUs was 84 (7 years × 12 sampling sites). The validity of the results was confirmed by comparison with other more traditional methods of identifying variables important for object classifications and by permutation tests of matrix correlation before and after removal of low-ranked and highly ranked species. Fuzzy clustering of Lake Simcoe SUs also revealed (*i*) the likelihood of trends in zooplankton community composition over the 7-year period and (*ii*) differences in composition possibly related to sampling-station depth. In particular, the shallowest sampling station in southern Cook's Bay had a zooplankton community structure that differed significantly from other stations during all years of the study. As a preliminary screening or data exploration tool, fuzzy clustering is particularly useful for analysis of ecological data.

Résumé : La classification floue assigne à chaque objet d'un dendrogramme un poids qui reflète le degré d'appartenance de l'objet à son groupe par rapport aux autres groupes. Lors d'une classification floue des taxons du zooplancton du lac Simcoe, un grand (720 km^2) lac d'eau dure d'Ontario, Canada, il a été possible d'ordonner tous les taxons selon leur contribution à la classification de l'unité d'échantillonnage (SU), où le nombre d'unités était de 84 (7 années × 12 sites d'échantillonnage). Ces résultats ont été validés par comparaison avec des méthodes plus traditionnelles d'identification des variables importantes dans la classification d'objets et des tests de permutation des matrices de corrélation, avant et après le retrait des espèces de rangs inférieurs et supérieurs. La classification floue des SU du lac Simcoe a aussi révélé (*i*) la vraisemblance des tendances dans la composition des communautés zooplanctoniques au cours des 7 années et (*ii*) la possibilité que les différences de composition soient reliées à la profondeur à la station d'échantillonnage. En particulier, la station d'échantillonnage la moins profonde dans la partie sud de la Baie de Cook avait une communauté zooplanctonique dont la structure différait de celle de toutes les autres stations au cours de toutes les années. La classification floue s'avère être un outil particulièrement utile pour le tri préliminaire et l'exploration des données écologiques.

[Traduit par la Rédaction]

Introduction

The results of a classical cluster analysis of ecological data usually include a graphical representation of the grouping (a dendrogram) of the sampling units (SUs) wherein those SUs grouped together in clusters are more similar in species compositions than SUs grouped in other clusters of the classification. A matrix of similarity or dissimilarity, i.e., distance, coefficients for all combinations of SUs calculated from species abundances at each SU generally serves as the starting point for cluster analyses, most of which are based on agglomerative hierarchical algorithms (e.g., Kaufman and Rousseeuw 1990).

One problem with this conventional so-called "hard-" or "crisp-" clustering approach is that it creates the dendrogram by assigning an object to only one cluster, so that once the object in question is assigned to a particular cluster, the fate of subsequent cluster assignments is more limited. A consequence of this apparent inflexibility is that the use of a different data transformation, a different method of calculating similarity (distance), or a different clustering strategy can result in a restructured dendrogram that may then lead to a different interpretation of the ecological data. There is, therefore, a level of uncertainty in the results of any hard-clustering exercise that requires the evaluation of a variety of objective data-analysis approaches in developing a consensus on the interpretation of data-analysis results.

Since the development of fuzzy logic more than 30 years ago (Zadeh 1965), there have been few applications of fuzzy

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cluster analysis to ecological data. Recent applications of fuzzy clustering in ecology (Equihua 1990; Marsili-Libelli 1991; Silvert 1997) have demonstrated certain advantages over traditional hard- or crisp-clustering strategies. Advantages relate to the inherent ability to identify overlapping and graded degrees of ecological affinity in a world where boundaries between ecological states are rarely well-defined. For example, Marsili-Libelli (1991) used fuzzy clustering to distinguish four trophic states (hypertrophic, eutrophic, mesotrophic, and oligotrophic) in a group of lakes with gradients in total phosphorus, chlorophyll, and water clarity. This was a particularly good demonstration of an appropriate use of fuzzy clustering, because the boundaries delimiting the trophic states of lakes are not sharply defined—one state grades into another.

In contrast to the hard-clustering methods, whereby an object is either a member of a cluster or it is not, fuzzy clustering generates graded cluster membership information. Fuzzy clustering assigns each object to each cluster by a membership weight or membership coefficient, where the sum of all membership coefficients = 1, thus providing a measure of the strength of the attachment of each object to its cluster. In multivariate analysis of ecological data, it is often important to determine which variables contribute most to the groupings of objects in cluster dendrograms or ordinations, where variables typically are species and objects are SUs. It occurred to us that the fuzzy cluster membership weights might be useful in defining the relative importance of variables (species), if the variables were themselves subjected to fuzzy clustering. The rationale for this is outlined in the Appendix.

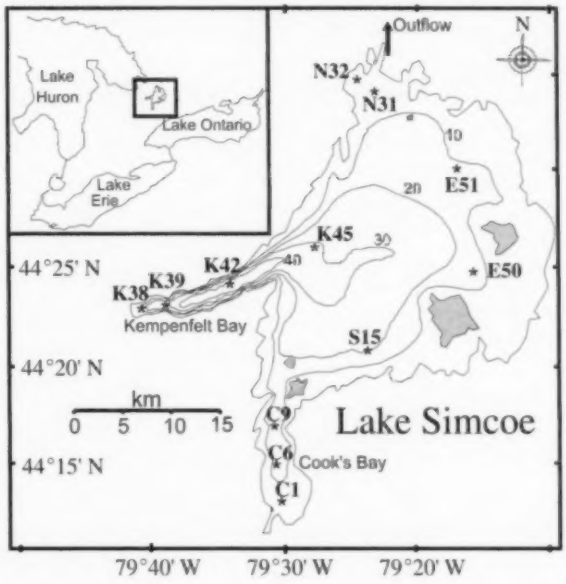
The main purpose of this paper is to propose the use of fuzzy cluster membership weights for ranking species important in a classification of SUs, using crustacean-zooplankton data from Lake Simcoe (Ontario, Canada) as a test case. Results are evaluated against two other more traditional methods for identifying variables important to object classifications. The other objective of this paper is to use the output of two fuzzy clustering and one traditional hard clustering strategies to identify elements of interannual (7 years) and spatial (12 sites) variability in the crustacean zooplankton of this large (720 km^2) hardwater lake. The data cover the period 1986–1992, prior to the establishment in 1994–1995 of populations of the spiny water flea (*Bythotrephes* sp.) and the zebra mussel (*Dreissena polymorpha*), two invading species. Companion papers (K.H Nicholls and C. Tudorancea, unpublished data) will describe other aspects of zooplankton community structure, including more detailed descriptions of spatial and temporal relationships among taxa and relationships with environmental variables and effects of invading species.

Materials and methods

Sampling and laboratory methods

Zooplankton samples were collected at approximately biweekly intervals at each of 12 Lake Simcoe stations (Fig. 1) during May–October of 1986–1992, after sites were located during the first spring sampling run with a "Loran C" navigation device and marked with buoys. Samples were taken from vertical tows, from 1 m above bottom to the lake surface, with a modified conical

Fig. 1. Lake Simcoe in southern Ontario, Canada, showing the locations of 12 sampling sites and the 10-, 20-, 30-, and 40-m depth contours. The lake has a mean depth of 14.2 m, a volume of 1.025×10^{10} m^3, and an area (excluding islands) of 722 km^2.



Clarke–Bumpus net (length 1 m, mouth diameter 12.5 cm, 80 µm Nitex mesh, diameter of net bucket 5.1 cm). At the lake surface, the net was submerged two or three times to rinse organisms down into the collection bucket from which the contents were washed into wide-mouthed jars containing a sugared (40 g sucrose·L^{-1}) 4% formalin solution dispensed from a washing bottle. A flow meter was not installed on the net in 1986. Net efficiencies for 1986 were set at a constant 95% (median value for 2 subsequent years). After 1986, net efficiencies were determined as the flow-meter reading from the sampling haul divided by the meter reading taken after the net was detached from the meter and hauled through the same vertical distance (McQueen and Yan 1993).

Identification, length measurement, and enumeration of organisms was done in a 10-mL gridded counting chamber on subsamples diluted in graduated cylinders. The dilution was set to achieve total counts of at least 250 specimens, made with a dissecting microscope fitted with a video camera and monitor. Length measurements were made with "Sprules-type" scissor-style callipers connected to a PC (Sprules et al. 1981). The associated software (Allen et al. 1994) incorporates the input enumeration and measurement data, combined with field data on net efficiency and length of net haul, to generate density and biomass estimates for each species. Nauplii and copepodite stages were enumerated but not identified to species in most cases (*Epischura lacustris* and *Senecella calanoides* copepodids were identified); separate categories for total calanoid and cyclopoid immature stages were included in the database and permitted estimates of their density and biomass. Taxonomic references included Dodson and Frey (1991) and Korovchinsky (1992), as well as those listed in Yan and Pawson (1995).

Data analysis

A SU is defined here as the average of one May–October period at one sampling site. Thus, in all, there were 84 SUs (12 sites × 7 years), each of which was designated according to the example "C186," where, in this case, C1 is the sampling site identifier (sta-

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Table 1. Rankings of all crustacean-zooplankton taxa for their importance in SU classification (all 12 Lake Simcoe sampling stations over the period 1986–1992, for a total of 84 SUs), according to three different methods.

Taxon	Rank 1	Rank 2	Rank 3
Acroperus harpae Baird	31	31	21
Alona spp.	28	27	26
Bosmina (Bosmina) longirostris (O.F. Müller)	1	5	8
Ceriodaphnia sp.	11	20	45
Chydorus sphaericus (O.F. Müller) s.l.	13	19	44
Daphnia ambigua Scourfield	41	41	17
Daphnia catawba Coker	29	35	33
Daphnia dubia Herrick, emend. Herrick	39	30	39
Daphnia galeata mendotae Birge	6	6	6
Daphnia longiremis Sars	15	17	42
Daphnia pulicaria Forbes emend. Hrbace	10	16	16
Daphnia parvula Fordyce	22	25	28
Daphnia retrocurva Forbes	5	8	2
Bosmina (Eubosmina) coregoni (Baird)	12	7	1
Eurycercus lamellatus (O.F. Müller)	30	34	20
Holopedium gibberum Zadach	23	18	43
Ilyocryptus spinifer Herrick	43	43	34
Leptodora kindtii (Focke)	26	23	38
Macrothrix laticornis (Jurine)	32	38	32
Pleuroxus hamulatus Birge	42	42	18
Sida crystallina (O.F. Müller)	24	28	25
Simocephalus serrulatus (Koch)	38	39	23
Simocephalus vetulus (O.F. Müller)	36	36	19
Bosmina (Eubosmina) longispina (Leydig)	34	33	22
Diaphanosoma birgei Kořínek	9	12	7
Daphnia sp. (unidentified)	20	22	40
Calanoid copepodid	4	2	14
Leptodiaptomus minutus (Lilljeborg)	16	10	5
Skistodiaptomus oregonensis (Lilljeborg)	14	14	11
Leptodiaptomus sicilis (Forbes)	21	15	12
Epischura lacustris Forbes	25	21	41
Senecella calanoides Juday	45	45	37
Calanoid nauplius	7	4	9
Cyclopoid copepodid	2	3	13
Diacyclops thomasi (Forbes)	17	9	3
Cyclops scutifer Sars	44	44	35
Acanthocyclops vernalis (Fischer)	18	24	29
Macrocyclops albidus (Jurine)	40	40	24
Mesocyclops edax (Forbes)	19	11	4
Orthocyclops modestus (Herrick)	35	37	30
Cyclopoid nauplius	3	1	15
Eucyclops serrulatus (Fischer)	27	26	27
Eucyclops neomacruroides Dussart & Fernando	37	32	36
Tropocyclops extensus Kiefer	8	13	10
Mesocyclops americanus Dussart	33	29	31

Note: Rank 1 is based on a Bray–Curtis hard clustering and was calculated from the contribution of each taxon to paired-cluster dissimilarities (Clarke (1993); the SIMPER routine in PRIMER); Rank 2 is from the sum of cluster centroids for each taxon (a *c*-means fuzzy clustering of SUs (Podani 1994)); Rank 3 is based on the cluster membership coefficients in a FANNY fuzzy clustering of taxa (Kaufman and Rousseeuw 1990; Appendix).

tion C1) and 86 identifies the year (1986). A SU × taxon matrix was constructed using all 84 SUs in the columns and 45 crustacean-zooplankton taxa (Table 1) in the rows of the matrix. The SU × taxon matrix was entered in SYN-TAX (Podani 1997), in which a $\log_{10}$ transformation preceded fuzzy clustering by the *c*-means algorithm. The matrix was also entered in PRIMER (Carr

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Table 2. Comparison of cluster memberships for a three-cluster classification of all 84 sampling units (SUs (site–years)) using a hard-clustering strategy and two different fuzzy clustering strategies.

(a) Bray–Curtis (group-average linkage) hard-clustering strategy.

Cluster	Size	SUs
I	8	C186[1], C187[1], C188[1], C189[1], C190[1], C191[1], C192[1], C691[1]
II	10	C986[3], E5086[3], E5186[2], K3886[2], K3986[3], K4286[3], K4586[3], N3186[3], N3286[3], S1586[3]
III	66	C686[1], C687[3], C688[3], C689[3], C690[3], C692[3], C987[3], C988[3], C989[3], C990[3], C991[3], C992[3]; E5087[3], E5088[3], E5089[3], E5090[3], E5091[3], E5092[3], E5187[3], E5188[3], E5189[3], E5190[3], E5191[3], E5192[3]; K3887[3], K3888[3], K3889[3], K3890[3], K3891[3], K3892[3], K3987[3], K3988[3], K3989[3], K3990[3], K3991[3], K3992[3], K4287[3], K4288[3], K4289[2], K4290[3], K4291[3], K4292[3], K4587[3], K4588[3], K4589[3], K4590[3], K4591[3], K4592[3]; N3187[3], N3188[3], N3189[3], N3190[3], N3191[3], N3192[3], N3287[3], N3288[3], N3289[3], N3290[3], N3291[3], N3292[3]; S1587[3], S1588[3], S1589[3], S1590[3], S1591[3], S1592[3]

(b) FANNY fuzzy clustering strategy (Kaufman and Rousseeuw 1990).

Cluster	Size	SUs
I	28	C186, C187, C188, C189, C190, C191, C192, C686, C687, C688, C689, C690, C691, C692, C987, C988, C989; E5088, E5092, E5188, E5191; K3887, K3888, K3889; N3188, N3288, N3290, N3292
II	27	C986, E5086, E5087, E5090, E5186, E5187, K3886, K3986, K3987, K3990, K3991, K3992, K4286, K4287, K4288, K4290, K4291, K4292, K4586, K4587, K4590, N3186, N3187, N3286, S1586, S1587, S1590
III	29	C990, C991, C992; E5089, E5091, E5189, E5190, E5192; K3890, K3891, K3892, K3988, K3989, K4289, K4588, K4589, K4591, K4592; N3189, N3190, N3191, N3192, N3287, N3289, N3291; S1588, S1589, S1591, S1592

(c) *c*-Means fuzzy clustering strategy (Podani 1994).

Cluster	Size	SUs
I	20	C186, C188, C189, C190, C191, C192, C690, C691, C692, C991; E5089, E5090, E5091, E5092, E5191, E5192; N3191, N3290, N3291, N3292
II	38	C187, C686, C687, C688, C689, C986, C987, C988; E5086, E5087, E5088, E5186, E5187, E5188, E5189; K3886, K3887, K3888, K3889, K3986, K3987, K3988, K4286, K4287, K4288, K4586, K4587, K4588; N3186, N3187, N3188, N3189, N3286, N3287, N3288, N3289; S1586, S1587
III	26	C989, C990, C992; E5190; K3890, K3891, K3892, K3989, K3990, K3991, K3992, K4289, K4290, K4291, K4292, K4589, K4590, K4591, K4592; N3190, N3192; S1588, S1589, S1590, S1591, S1592

Note: For the two fuzzy classification methods, partitions were defined by the highest fuzzy cluster membership coefficients for each SU. The superscripts 1, 2, and 3 on the SUs of the classification in *a* (e.g., C186[1]) indicate to which of three new clusters the SU can be assigned on the basis of consensus fussy partition analysis (Podani 1994; i.e., a consensus partition best representing all three classifications).

1997), for calculation of Bray–Curtis similarities after square root transformation of the data and clustering by a group-averaging algorithm. The resulting matrix was converted to a matrix of dissimilarities for entering in the PC fuzzy clustering program FANNY (Kaufman and Rousseeuw 1990). For both fuzzy clustering programs, we specified a three-cluster output, because the dendrogram produced by the Bray–Curtis method suggested three well-defined SU clusters.

In an attempt to reconcile the differences in dendrogram structure among the three outputs (two fuzzy partitions and one hard partition), a fuzzy consensus partition was sought that would best summarize these three dendrograms. First, the two fuzzy classifications were converted to hard partitions on the basis of their highest membership coefficients. The cluster memberships of the three dendrograms were then summarized as cluster-membership vectors, each consisting of an array of values coded 1, 2, or 3 (representing membership in one of three clusters) for each dendrogram. A partition consensus algorithm was applied that examines all possible fits of clusters and makes a final selection based on the fit that produces the minimum sum of squared differences between the corresponding membership coefficients (Podani 1994). Output was a fuzzy clustering that was converted to a hard partition using the highest fuzzy consensus membership coefficients to assign SUs to their new consensus clusters.

The importance of taxa in defining SU clusters was determined in three different ways. (1) The percentage contribution by each taxon to the average between-cluster dissimilarity was calculated (Clarke 1993; Carr 1997). Because this produced a separate rank-

Fig. 2. The two-group solution for a fuzzy clustering (*c*-means method) of all 84 Lake Simcoe SUs, using all 45 zooplankton taxa listed in Table 1.

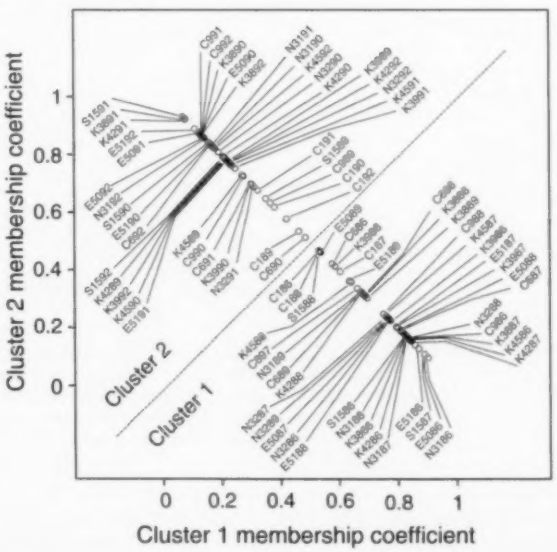

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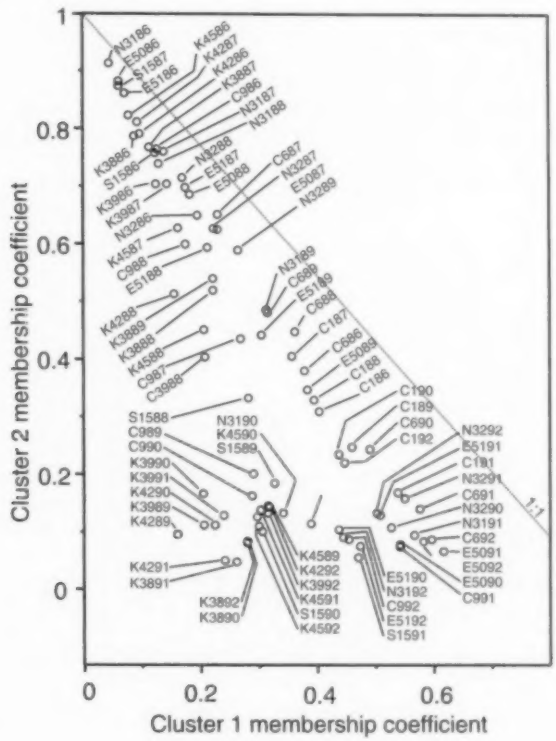
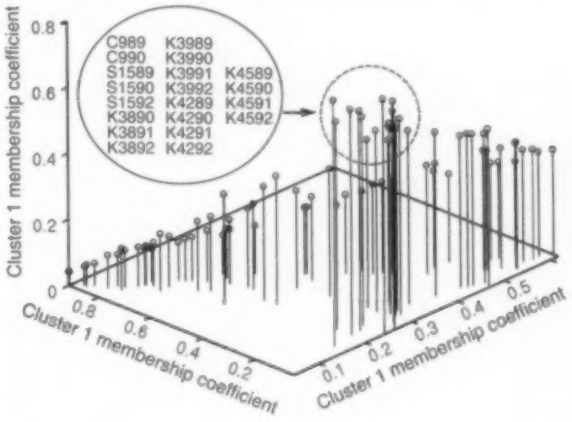
Fig. 3. The three-group solution for a fuzzy clustering (*c*-means method) of all 84 Lake Simcoe SUs, using all 45 zooplankton taxa listed in Table 1; only the first two cluster memberships are plotted.



ing of all taxa contributing to each cluster pair (i.e., clusters 2 and 1, 3 and 1, and 3 and 2), an overall ranking of the taxon importance in overall dendrogram structure was achieved by simple averaging. Not all taxa were common to all cluster pairs, however (i.e., a few taxa were unranked for one or more of the cluster pairs, because they did not contribute to the average between-cluster dissimilarity); these unranked taxa were assigned a rank of $n + 1 = 46$, where $n = 45$ taxa in total. (2) The *c*-means fuzzy SU clustering produced a table of variable (taxon) contributions to the centroid of each of the three clusters that were totalled for each taxon and sorted in descending order to provide scoring for all 45 taxa. (3) A FANNY clustering of 4th root transformed Bray–Curtis dissimilarities of all taxa produced a table of fuzzy cluster membership coefficients that were sorted in descending order for each taxon; then, differences between successive membership coefficients were summed (Appendix).

For comparison of the efficacy of each of the three separate rankings of taxa produced, each ranked list was subsequently divided at the middle rank and new matrices of Bray–Curtis dissimilarity values for all SUs were constructed using (*i*) the highest ranked 50% of taxa and (*ii*) the lowest ranked 50% of taxa, in each of the three rankings. This generated six new dissimilarity matrices, each of which was compared by correlation (where $n = 84 \times 84 = 7056$) with the original matrix based on all 45 taxa. A high correlation between the matrix of the top-ranked 50% of taxa and the original matrix and a corresponding low correlation between the matrix of the low-ranked 50% of taxa and the original matrix indicates a successful ranking of taxa and that the 45 taxa used in this study differed in their ability to discriminate SU clusters. Differences in the correlations between the original matrix and the top 50% of ranked taxa and the original and bottom 50% of ranked taxa indicate relative differences in the adequacy of three taxon-ranking procedures. Traditional tests of statistical difference cannot be based on these correlations because the elements of the two matrices being compared are not independent (50% of the taxa were used to generate SU dissimilarities in the two matrices being compared). Statistical difference was therefore inferred from Mantel-type permutation tests (Dietz 1983), in which simulated correlation coefficients were produced after randomly reordering 10 000 times the rows of one of the matrices being compared. The actual measured correlation coefficient was compared with the statistical distribution of the simulated correlation coefficients, including the tail probabilities, for inference of the statistical significance of the measured value.

Fig. 4. The three-group solution for a fuzzy clustering (*c*-means method) of all 84 Lake Simcoe SUs, using all 45 zooplankton taxa listed in Table 1, plotted in three dimensions.



Results

Fuzzy definition of SUs

Only the *c*-means clustering of the 84 Lake Simcoe zooplankton SUs is presented here in detail, but a summary of cluster memberships for all three classifications (one hard and two fuzzy partitions) is presented in Table 2. The fuzzy clustering of the 84 SUs produced a two-group solution, with cluster membership coefficients revealing some temporal structure (Fig. 2). Cluster 1 membership coefficients were highest for SUs of the early sampling period (1986 and 1987), while cluster 2 was defined by those SUs representing the latter years of the study (1991–1992). SUs from the intermediate years (1988–1990) were represented in both clusters, thus suggesting the presence of a time trend worthy of further data analysis. Some exceptions to this distribution of SUs between the two clusters include Station C1 for 1986 and 1987 and Station C6 for 1986; these have their highest membership coefficients in cluster 1, but also have significant cluster 2 weights (Fig. 2). This suggests that a higher level of cluster segregation (three or more clusters) might lead to separation of some of the C1 SUs into distinct clusters.

The three-group fuzzy clustering solution does add new insights for interpreting the distribution of the Lake Simcoe

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Fig. 5. The three-group solution (clusters I, II, and III) for a hard partition of all 84 Lake Simcoe SUs, using all 45 zooplankton taxa listed in Table 1. Six subgroups of cluster III (*a–f*) are further identified at an inter-SU similarity level of about 85%, based on the Bray–Curtis measure of similarity and a group average clustering strategy.

SUs. In a two-dimensional placement of all SUs, in which the two axes are again membership coefficients in two of the clusters, the deviation from the 1:1 diagonal is an indicator of the relative magnitude of the third cluster membership (Fig. 3). Thus we see that, again, the 1986 and 1987 SUs are concentrated at one end of the plot (in this case, having relatively low cluster 1 membership coefficients and relatively high cluster 2 coefficients), but that these SUs also have relatively low cluster 3 membership coefficients, as inferred by the small deviations from the 1:1 line. In contrast, those SUs with the highest membership weights in fuzzy cluster 1 are now showing a high tendency to affiliate with new fuzzy cluster 3 (high deviations from the 1:1 line; Figs. 3 and 4). The most noticeable new interpretation provided by the three-cluster solution is the presence of a well-defined third cluster containing many SUs from the K series of sampling stations, along with some S15 and C9 SUs (Fig. 4), all of which are deepwater sites. So, a three-group fuzzy clustering of SUs suggests the influence of some temporal as well as other intersite differences that may be related to station depth.

Hard-partition definition

There were both similarities and differences between the patterns revealed by the fuzzy cluster analyses and those suggested by conventional hard clustering. Clusters I and II of the hard classification (Fig. 5) were very well defined. All station C1 SUs were assigned to cluster I. C691 was the only other SU assigned to cluster I (Fig. 5; Table 2). Ten of the 12 sampling stations sampled in 1986 were assigned to cluster II. Besides C186 in cluster I, only C686 was excluded from cluster II and assigned to cluster III_b, where it showed higher similarity with station C6 SUs from other years. Had C691 been assigned to group III_b instead of cluster I, then cluster I and cluster III_b would have been the exclusive domain of the C1 and C6 SUs, respectively, with the exception of C987 in cluster III_b. This might have been anticipated from the fuzzy cluster membership coefficients, which showed that C686 was not strongly affiliated with any of the three fuzzy clusters (coefficients of 0.38, 0.38, and 0.24). Similarly, C691 could not be assigned to any of three clusters in a consensus fuzzy clustering (see below), with consensus membership coefficients of 0.33 for each of the new consensus clusters.

Cluster III of the hard partition was composed of six groups at about the 85% similarity level, two of which, III_a and III_d, contained only two and three SUs, respectively, all representing the 1990 sample year (Fig. 5). Clusters III_c, III_d, and III_e contained a heterogeneous aggregation of SUs representing all stations except C1, C6, and K42. Patterns in SU distribution among the three clusters relating to lake location, station depth, or year are not immediately apparent. Cluster III_f was dominated by the K series of sampling stations and, with the exception of E5192, the other stations assigned to cluster III_f (one C9 and three S15 SUs) may indicate some affinity to greatest water depth. There is close

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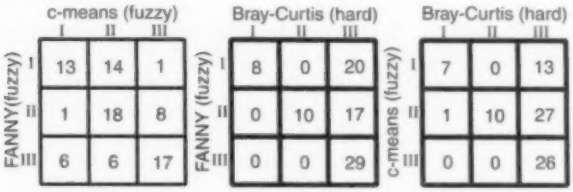
Fig. 6. Two-way contingency tables for comparisons of the cluster memberships of the three different classifications, where I, II, and III represent the clusters for a three-group solution in each case.



agreement between the composition of this group and the group of 20 SUs identified in Fig. 4 that had the highest membership coefficients for the third fuzzy cluster.

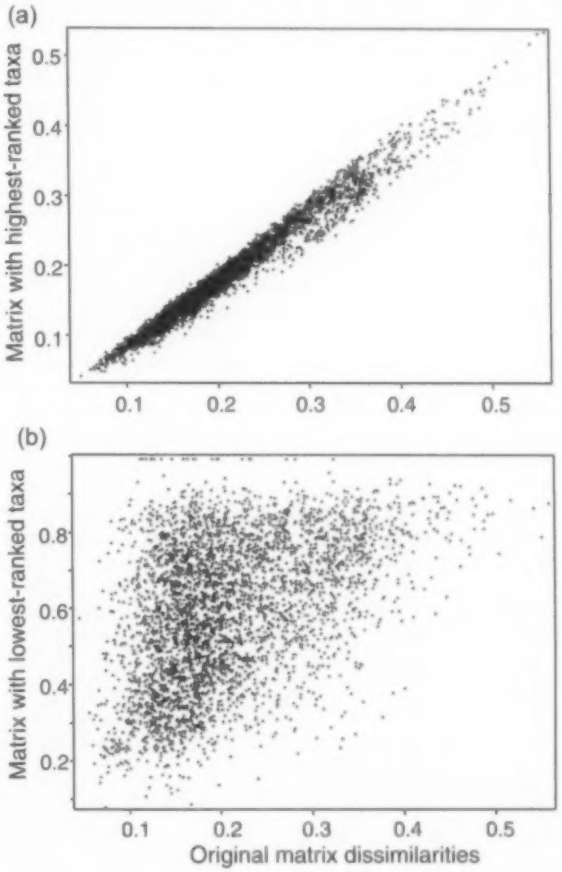
The cluster memberships of the three different classifications can be compared (in pairs) in a two-way contingency table, in which the rows represent the clusters from one classification and the columns represent the clusters from the other classification (Fig. 6). The entries are the number of SUs common to both. Perfect agreement between two classifications would result in zero values for all off-diagonals. A measure of the concordance between two classifications is the proportion of the total number of SUs contributed by the sum of the diagonal entries. The highest concordances were for the FANNY fuzzy versus *c*-means fuzzy comparison and the FANNY fuzzy versus Bray–Curtis hard comparison at 57 and 56%, respectively. The *c*-means fuzzy versus Bray–Curtis hard comparison was only slightly lower at 51% (Fig. 6).

In view of the differences among the three classifications, a consensus fuzzy partition was developed (see Materials and methods), to best capture the "average" structure encompassed in the two fuzzy partitions and one hard partition. These new cluster assignments were made on the basis of their fuzzy cluster membership coefficients and were included in Table 2*a* as superscripts identifying the SU as a member of new cluster I (SU^1), new cluster II (SU^2), or new cluster III (SU^3). When assigned to new clusters on the basis of their highest membership coefficients, the new fuzzy consensus classification contained SUs C186, C187, C188, C189, C190, C191, C192, and C686 in cluster I. Cluster II contained only E5186, K3886, and K4289, while consensus cluster III contained all remaining SUs. Only one SU (C691) could not be assigned unconditionally to a new consensus cluster, because it lacked unique maximum weight, with values of 0.333 for each of the three new clusters. For completeness, it was arbitrarily assigned to new cluster I. The new consensus fuzzy partition most resembled the Bray–Curtis hard partition. Concordances between the new consensus partition and the three original classifications (FANNY fuzzy, *c*-means fuzzy, and Bray–Curtis hard) were 45, 39, and 87%, respectively.

Importance of variables (taxa) in cluster definition

Of the three methods used to rank taxa for their contribution to SU classifications, the centroid method from the *c*-means fuzzy clustering (Rank 2) and Clarke's (1993) method for Bray–Curtis based hard clusters (Rank 1; henceforth termed the SIMPER method) achieved rankings that were very similar (Table 1). The Spearman rank correlation coefficient for these two ordered taxa lists was 0.948 ($P < 0.0001$). Rank 3 (from fuzzy clustering of taxa) pairings with Rank 1 and Rank 2 produced much lower Spearman rank correlation coefficients of 0.475 and 0.540, respectively, although both were statistically significant ($P < 0.001$). Some obvious disagreements between rankings included the following: (1) in both Rank 1 and Rank 2, *Pleuroxus hamulatus* occupied the 42nd position, while in Rank 3 it occupied the 18th position; (2) the "unidentified *Daphnia*" category placed near the bottom in Rank 3 (40th) but placed 20th and 22nd in Rank 1 and Rank 2, respectively; (3) *Diacyclops thomasi* ranked 17th, 9th, and 3rd and *Mesocyclops edax* ranked 19th, 11th, and 4th, in Rank 1, Rank 2, and Rank 3, respectively, while *Daphnia longiremis* ranked 15th, 17th, and 42nd in Rank 1, Rank 2, and Rank 3, respectively (Table 1). A few taxa were assigned relatively high ranks by all three methods. For example, *Daphnia galeata mendotae* was ranked 6th by all three methods, while *Bosmina longirostris* was ranked 1st, 5th, and 8th and

Fig. 7. Correlation of the original SU dissimilarity matrix developed using all 45 zooplankton taxa with new matrices developed using (*a*) the top-ranked 50% of taxa and (*b*) the bottom-ranked 50% of taxa. Taxon rankings were based on membership coefficients from a fuzzy clustering of taxa.



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Daphnia retrocurva was ranked 5th, 8th, and 2nd by the Rank-1, Rank-2, and Rank-3 methods, respectively (Table 1).

As a test of the validity of the three different taxon rankings, new Bray–Curtis dissimilarity matrices of the Lake Simcoe zooplankton SUs were calculated (*i*) after removing the lowest-ranked 50% of taxa and (*ii*) after removing the highest-ranked 50% of taxa. Each of these new matrices was compared with the original matrix (all 45 original taxa), both in a correlation analysis and in a Mantel-type permutation test, to determine statistical significance. After removal of lowest-ranked taxa, valid taxon rankings should produce new matrices that are much better correlated with the original matrix than those from which the top-ranked taxa were removed. This was the case for all three of these rankings; to save space, however, only the results from Rank 3 are illustrated (Fig. 7). In this case, the correlation coefficient for the correlation between the original matrix and the new matrix containing only the top-ranked taxa was 0.986, in contrast with a correlation coefficient of only 0.432 for the correlation between the original matrix and the new matrix containing only the bottom-ranked taxa (Fig. 7). Corresponding correlation coefficients for Rank 1 were 0.998 and 0.207, and for Rank 2 were 0.995 and 0.141, for matrices resulting from the removal of bottom-ranked and top-ranked taxa, respectively. Permutation tests showed that, for all three taxon rankings, none of the matrices containing only the top-ranked 50% of taxa was statistically different from the original matrix that employed all taxa. Neither were any of the new matrices using top-ranked taxa statistically different among themselves. For example, the calculated correlation coefficient for the comparison of the SU matrices built using the top-ranked Rank-1 and Rank-3 lists was 0.990. This was well outside the distribution of simulated correlation coefficients resulting from 10 000 random re-orderings of matrix rows, in which the mean was –0.001 and the maximum was only 0.315. The probability that the observed correlation coefficient of 0.990 could have resulted from chance is, therefore, remote.

Discussion

In this paper, we have shown that, when fuzzy clustering is applied in a conventional way, simply as a tool to distinguish among SUs represented by many different taxa, it offers insights that allow hypothesis testing at higher levels of experimentation and data analysis. For example, the two- and three-group fuzzy clustering solutions revealed the likelihood of temporal trends and intersite differences in zooplankton species composition that may be related to station depth. The restriction of membership in the first consensus cluster to the C1 SUs (all 7 years), along with the assignment of 95% of the remaining SUs to the new consensus cluster III, has emphasized the distinct character of Station C1, and further suggests apparent zooplankton compositional differences at the deepwater stations (the K series of stations, along with S15 and C9). As well, the temporal differences suggested in the original fuzzy clusterings were less important in defining Lake Simcoe zooplankton community structure than the differences imparted by the disparity between station C1 and the remaining stations. Thus, as a preliminary-screening or data-exploration tool, fuzzy clustering is particularly useful at the early stages of ecological-data analysis.

Among the few existing examples of fuzzy clustering of ecological data, Marsili-Libelli (1991) provided several, including (1) distinguishing among three species of iris, (2) distinguishing a crop species grown under stress (defoliation) from crops grown normally, (3) classifying four benthic invertebrate species among three different habitats, (4) classifying a group of soils in northern Italy characterized by chemical and meteorological parameters, and (5) identifying trophic-state gradients in lakes. Other recent applications of fuzzy set theory in ecology include the development of a procedure for "fuzzy coding" of biological and environmental data prior to processing by correspondence analysis (Chevenet et al. 1994). Silvert (1997) suggested that fuzzy set theory could be used for developing new ways of evaluating the exceeding of industrial effluent discharges. Recently, Mackinson et al. (1999) described how fuzzy set theory could be used to describe uncertainty in fish stock–recruitment data, an approach that takes advantage of data-poor situations where more traditional protocols of data analysis may be inappropriate. They identified five other fisheries–resource management areas of potential application for fuzzy set theory. Clearly, the scope for application of fuzzy classification in ecology is wide and varied.

In this paper, we have also demonstrated a new application for fuzzy cluster analysis of ecological data, whereby species can be ranked for their contribution to the structure of a SU dendrogram. This is important, because there are few good ways to do this quantitatively (Clarke 1993). Previously used techniques for evaluating the importance of taxa in the structure of sampling-site classifications include nodal analysis and related methods (Lambert and Williams 1962; Lyche 1990; Flores and Barone 1994) and calculations of the percentage contribution each species makes to the average dissimilarity between clusters (Clarke 1993), both of which, in the past, have been applied to hard clusters only. *c*-Means fuzzy clustering of objects determines the contribution of each variable to the centroid of each object cluster and, thus, offers another way of identifying important discriminating species when objects are SUs and variables are species. Equihua (1990) exploited this feature to identify those species of terrestrial plants that contributed most to the cluster definition of mooreland communities, and we used this method to generate our Rank-2 ordering of Lake Simcoe zooplankton taxa.

Using the cluster membership weights from Kaufman and Rousseeuw's (1990) fuzzy clustering algorithm FANNY appears to be another effective way to rank taxa (our Rank 3) for their contribution to SU classification. As shown here, fuzzy cluster membership coefficients for taxa can be expressed mathematically (as the sum of paired differences) and used to rank taxa for this purpose. Although there are undoubtedly more thorough ways to demonstrate this, removal of the top-ranked and bottom-ranked 50% of the taxa to generate two new SU dissimilarity matrices for comparison with the original matrix was a simple demonstration of the utility of the basic approach. More detailed approaches could involve, for example, the sequential removal of the lowest-ranked taxa and multiple SU matrix comparisons, to reveal the minimal number of taxa that "survive" the thin-

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ning process and still generate a SU dissimilarity matrix that is statistically indistinguishable from the original (with statistical significance inferred by permutation tests).

Even at the simple level of evaluation used here (using a 50% cut-off), it is clear that a large number of Lake Simcoe zooplankton taxa (>50% of the original total number of 45) played a minimal role in defining site–year zooplankton community dissimilarities. This has important practical implications for establishing an optimal level of effort in ongoing monitoring programs, in that it lets us know which taxa need special attention in the identification and enumeration of zooplankton samples and which can perhaps be ignored if time and funding for the work are at a premium. More importantly from an ecological perspective, a short list of ecologically informative taxa can simplify the development of (1) hypotheses relating to species interactions (predation and grazing) and (2) models of environmental effects on temporal trends in a few "highly ranked" taxa.

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Appendix

Fuzzy clustering algorithms and membership coefficients

There are three fuzzy clustering algorithms in use. One is the fuzzy *k*-means or *c*-means algorithm (Bezdek 1987), which has analogy to the common *k*-means method of nonhierarchical hard clustering, because it minimizes the so-called fuzzy sum of squares of clusters based on the Euclidean distance between an object and the centroid of its cluster. This algorithm is used in the PC computer program SYN-TAX (Podani 1994). The others are the MND2 algorithm of Roubens (1978) and the FANNY algorithm of Kaufman and Rousseeuw (1990). The differences between the latter two is that FANNY minimizes the fuzzy equivalent of a sum of error functions for each cluster, while MND2 minimizes the fuzzy equivalent of a sum of products of the number of objects times the error functions. This subtle difference apparently biases MND2 results towards clusters with more equal number of objects and error functions (Kaufman and Rousseeuw 1990). Advantages of the FANNY algorithm over the fuzzy *c*-means algorithm include

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its lower sensitivity to outliers and a better recognition of nonspherical clusters (Trauwaert 1987).

Output from fuzzy clustering typically includes tables of membership weights or coefficients (Kaufman and Rousseeuw 1990; Podani 1994) that reveal how tightly an object is linked to its cluster relative to its affinities to other clusters. Metrics based on membership weights (Dunn's partition entropy, Bezdek's partition coefficient, coefficient of overall separation, silhouette coefficient) allow ways of defining an optimal number of clusters (Kaufman and Rousseeuw 1990; Podani 1994) and apparently have no analog in commonly used hierarchical agglomerative ("hard" or "crisp") clustering methods. The graded cluster memberships produced by fuzzy clustering are intuitively applicable to ecological problems, because of the presence of "noise" in many relationships among environmental–ecological variables.

Our proposed use of fuzzy cluster membership weights for ranking taxa for their contribution to sampling unit (SU) classification structure is based on fuzzy clustering of taxa, where the method utilizes all possible pairs of species for which similarity or distance values have been calculated. In an ecological context, the calculated index of similarity for two species will be high, if these species are present at similar abundances at the same sites. Species that form well-defined clusters should therefore have higher levels of similarity among themselves than with species assigned to other clusters. In this clustering of species, those species that have relatively low affinity for the cluster to which they are assigned are unlikely to be good discriminators of SU clusters, because of their affinity to link with more than one species cluster. Fuzzy clustering allows these affinities to be quantified. Species with high membership coefficients in a species cluster indicate a high affinity for that cluster, and species with high membership coefficients in a cluster will all be significantly represented at the same set of sites. Similarly, species with low membership coefficients for a cluster can also be good indicators (providing they have high membership weights in other clusters) by virtue of their exclusion from some sites and domination at others. Both groups of species will be good discriminating species and, thus, contribute significantly to site-to-site differences in community structure. Those species with membership coefficients approaching $1/n$ for all clusters, where n = the number of clusters, will be the poorest discriminators of sites, because of their equally low degree of assignment to all clusters. Thus, it should be possible to use the fuzzy cluster membership coefficients from a species dissimilarity matrix to identify those species contributing most to inter-SU differences, after suitably ordering the sum of the differences in species cluster membership weights as described below.

Table A1. Fuzzy cluster membership coefficients for three taxa (i, j, and k) among three clusters, for demonstration of the calculation of taxon ranks.

	Fuzzy cluster membership weight		
	Cluster A	Cluster B	Cluster C
Taxon i	0.333	0.333	0.333
Taxon j	0.700	0.290	0.010
Taxon k	0.700	0.150	0.150

Consider a three-cluster solution of a fuzzy clustering of three taxa, i, j, and k only. Membership coefficients of taxon i in clusters A, B, and C are 0.333, 0.333, and 0.333. Corresponding fuzzy cluster membership coefficients for taxon j are 0.7, 0.29, and 0.01, and for taxon k are 0.7, 0.15, and 0.15 (Table A1). Taxon i is the least valuable discriminator of the clusters, because it shows no affinity for any cluster (equal membership coefficients for all three clusters). If ranked simply on the basis of highest cluster membership coefficient, taxon j and taxon k are tied, because their highest coefficient (for cluster A) is 0.7 in each case. However, taxon k shows equal affinity for clusters B and C, while taxon j shows a higher affinity for cluster B and a lower affinity for cluster C than does taxon k. Taxon j should therefore be a better overall discriminator than taxon k. Quantitatively, this is demonstrated by summing the differences in their ordered membership coefficients. So, the score for taxon i is $(0.333 - 0.333) + (0.333 - 0.333) = 0$; the score for taxon j is $(0.7 - 0.29) + (0.29 - 0.01) = 0.69$; and the score for taxon k is $(0.7 - 0.15) + (0.15 - 0.15) = 0.55$.

The output from a FANNY fuzzy clustering of 4th root transformed Bray–Curtis dissimilarities of all 45 Lake Simcoe crustacean-zooplankton taxa produced a table of fuzzy cluster membership coefficients. Scores were determined as described above for all 45 Lake Simcoe taxa, then sorted in descending order to generate a species ranking ("Rank 3" in Table 1).

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Seston supply to sea scallops (*Placopecten magellanicus*) in suspended culture

C.A. Pilditch, J. Grant, and K.R. Bryan

Abstract: The filtration activity of dense aggregations of bivalves can locally reduce the concentration of seston, potentially limiting production. Under these conditions, the currents that supply seston cannot offset ingestion by the bivalves and the carrying capacity is exceeded. We conducted a field and modelling study to examine factors affecting seston supply to a culture of suspended sea scallops (*Placopecten magellanicus*) in Whitehaven Harbour, Nova Scotia. Even though seston flux to the scallops was reduced by 40% due to the attenuation of flow by culture gear, a reduction in seston concentration was not observed. The field data were then used to parameterize a quasi two-dimensional advection–diffusion model that quantified the relationship between the tidally driven seston supply and consumption by scallops. The model predicted a minimal reduction in seston concentration (<5%) within the lease (80 × 50 m), consistent with field observations. However, expanding the lease to occupy the area available for cultivation (1000 × 250 m) reduced the seston concentration in the centre of the lease by 20–50%, potentially limiting growth. Modelling results emphasized that by altering the lease geometry (lease width to length ratio), the supply of seston can be optimized.

Résumé : Les activités de filtration opérées par de grands rassemblements de bivalves peuvent, à l'échelle locale, réduire la concentration du seston et, ainsi, potentiellement limiter la production. Dans de telles circonstances, les courants qui assurent la distribution du seston n'arrivent pas à compenser l'ingestion par les bivalves et le stock limite est alors dépassé. Des études sur le terrain et des modélisations ont permis d'étudier les facteurs qui affectent l'approvisionnement en seston d'une culture de pétoncles (*Placopecten magellanicus*) en suspension à Whitehaven Harbour en Nouvelle-Écosse. Bien que l'obstruction par l'équipement d'élevage ait diminué de 40% le flux du seston vers les pétoncles, il n'y avait pas de réduction de la concentration de seston. Les observations de terrain ont ensuite été intégrées dans un modèle d'advection–diffusion quasi-bidimensionnel qui permet de quantifier la relation entre l'approvisionnement du seston par la marée et la consommation par les pétoncles. La modèle prédit une réduction minime de la concentration du seston (<5%) à l'intérieur de la concession d'élevage (80 m × 50 m) qui correspond aux observations. Cependant, si on élargissait la concession de manière à couvrir toute la surface propre à l'élevage (1000 m × 250 m), on réduirait la concentration du seston dans le centre de la concession de 20–50%, limitant ainsi potentiellement la croissance. Les prédictions du modèle illustrent comment, en modifiant la géométrie de la concession (largeur:longueur), on peut obtenir un apport optimal de seston.

[Traduit par la Rédaction]

Introduction

In recent years, the culture of marine bivalves has increased rapidly as part of a worldwide growth in aquaculture industry (Boghen 1995). For expansion to continue at this rate, either new sites will need to be developed or the intensity of cultivation within existing sites increased. Successful management of existing sites and the selection of suitable new sites depends on good estimates of the environmental carrying capacity, i.e., the stocking density at which production levels are maximized without negatively affecting growth rates (Carver and Mallet 1990). To accomplish this goal, recent simulation models of bivalve culture sites have brought together aspects of the dynamics of food supply (such as seasonal cycles of phytoplankton production and resuspension) with the physiological energetics of the cultured species in order to predict growth under field conditions (e.g., Grant et al. 1993; Dowd 1997). The resulting models estimate carrying capacity by increasing the bivalve density until intraspecific competition for food reduces growth (Grant et al. 1993; Dowd 1997). However, these models are limited in their ability to predict carrying capacity because they ignore the important role that flow plays in supplying food to the bivalves.

In dense aggregations of bivalves, the concentration of particles available for ingestion depends on the rate of particle replenishment by the currents. Natural beds of filter-feeding bivalves are known to substantially deplete particle concentrations in the overlying waters during periods of low flow (Fréchette et al. 1989). Under these conditions, the currents that supply seston and generate the turbulent exchange between depleted boundary layers and those higher in the water column cannot offset ingestion by the bivalves. Re-

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duced seston concentrations during periods of low flow have been proposed as a mechanism to explain spatial gradients in growth within bivalve beds (e.g., Fréchette and Bourget 1985; Newell 1990). At high flows, seston supply exceeds the rate of consumption and food availability depends only on ambient particle concentration (Wildish and Kristmanson 1997). The relationship between the flow-driven seston supply and its consumption by bivalves is also likely to be a critical factor determining the production of suspended cultures (Ryther 1969; Incze et al. 1981).

In suspended culture, the rate at which food is supplied to the bivalves can potentially limit growth (exceed the carrying capacity) at three different scales. At the small scale, various studies (e.g., Parsons and Dadswell 1992; Côté et al. 1994) have determined the optimal stocking density of the culture unit (e.g., individual pearl or lantern net). At intermediate scales, there is the possibility of depletion occurring within the lease as water passes from one culture unit to the next, and, finally, at bay scales, seston-depleted water exiting one lease may feed into another lease downstream (Grant et al. 1993). Far fewer studies have addressed carrying capacity at these larger scales. Evidence suggests that at least at the site scale, seston depletion is a significant factor affecting production. For example, chlorophyll concentrations exiting mussel rafts were reduced by 53–64%, and significantly lower growth occurred for individuals positioned on the downstream side of a lease (Manson 1976; Navarro et al. 1991). Assuming that aquaculturists will optimize the stocking density at the scale of the culture unit, then the relationship between bivalve consumption and the food supplied by the currents at the lease scale will determine the carrying capacity of a site. Moreover, the dependence on an advected food source means culture sites selected solely for a high seston quality/quantity may actually have a low carrying capacity because of insufficient flow rates (Ryther 1969).

We undertook a field and modelling program to examine the factors affecting the seston supply to a culture of suspended sea scallops (*Placopecten magellanicus*) in Whitehaven Harbour, Nova Scotia. Using optical instruments and current meters, we measured the temporal variation in seston concentration and flow velocity to determine the dominant processes affecting food supply to the lease in late summer. During two tidal cycles, scallop feeding rates were measured and seston concentration monitored inside and outside the lease to see whether depletion occurred in periods of low flow. Processes affecting the seston concentration within the lease were then quantified with a simple model that examined the interplay between the current-driven seston supply and consumption by the scallops. While this relationship has been well quantified in modelling studies for benthic suspension-feeding bivalves (e.g., Fréchette et al. 1989), the same is not true for bivalves in suspended culture. In the only previous study, Incze et al. (1981) used an algebraic model to calculate the removal of seston as water flowed through successive tiers of a mussel raft culture. Here, we extend their model to include temporal variations in the flow velocity as well as additional sources of seston; specifically, an across-lease exchange term is considered to predict the optimal shape (length to width ratio) that minimizes seston depletion for a given set of flow conditions.

Methods

Study site

Whitehaven Harbour is located on the northeast coast of Nova Scotia in Guysborough County (Fig. 1). The bay is approximately 6 km long and 1–2 km wide and comprises deepwater channels (down to 26 m) and many islands, some of which are connected at low water. Tides are semidiurnal with a mean range of 1.4 m.

Sea scallops are grown in the northwest part of the bay at an experimental site 0.5 km southeast of Munroe Pt. where the mean water depth is 19 m. At the time of study, the area under cultivation measured 80 × 50 m, a small fraction of the total lease (1000 × 250 m) available for cultivation (Fig. 1). The cultivated area contained 10 longlines rigged with vertically hanging ropes (drops) spaced 1 m apart. Scallops were glued back-to-back on narrow strips of mesh (1.5 m in length, 20 scallops per strip); each strip was attached at one end to a hoop (0.5 m in diameter, six strips per hoop). Each vertical drop consisted of five hoops separated so that the scallops occupied a depth range between 5 and 15 m above the bottom. The site was stocked with three longlines of adult scallops (70–100 mm shell height, 3 years old) and seven lines of juveniles (40–50 mm shell height, 2 years old). The average scallop density within the occupied portion of the lease was 12.2 individuals·m^{-3}.

Field measurements of seston supply and demand

The transport rate and concentration of seston entering the lease were determined using an instrument array moored 10 m above bottom (midculture depth) at a reference site (RS, mean water depth = 19 m) 100 m to the southwest of the lease (Fig. 1). The array consisted of an S4 electromagnetic current meter and a SeaTech fluorometer and transmissometer (10-cm path length) connected to an Applied Microsystems ECM-12 data logger. Estimates of current velocity, temperature, and salinity were recorded every 10 min and the concentration of chlorophyll *a* (Chl *a*) and total suspended particulate matter (SPM) every hour during August 1995. An additional S4 was placed at the centre of the lease (LS) (Fig. 1) to establish whether the presence of culture gear influenced the flow velocity and thus the flux of seston to the scallops.

To determine whether scallop feeding influenced the food availability within the lease, vertical profiles of Chl *a* concentration at LS and RS were compared. Chl *a* is an appropriate tracer due to its preferential ingestion by *P. magellanicus* (MacDonald and Ward 1994) and the concentration is easily determined. Measurements were made at 0.5-m intervals between the surface and 2 m and then at 1-m intervals down to 18 m with a profiling instrument that consisted of a thermistor and a fluorometer connected to a LI-COR LI1000 data logger. A single profile took 20–25 min to complete; thus, each site was sampled every 45–50 min for a complete tidal cycle on 9 and 10 August 1995. During profiling, the sampling interval of the moored fluorometer was decreased to 5 min to allow instantaneous comparisons of Chl *a* concentration at RS and LS.

The transmissometer and fluorometers were calibrated in situ by comparing the logged voltage data with water samples collected by a hand-operated bilge pump within 50 cm of the instrument and analysed for Chl *a* and SPM. Water was prefiltered through a 120-μm Nitex mesh to eliminate the variance associated with flocs. The concentration of SPM was determined by filtering replicate water samples through preashed (450°C for 2 h) and weighed (±0.01 mg) GF/C filters (nominal pore size 1.2 mm). Salt was removed from the filters by rinsing with 20 mL of isotonic ammonium formate. Chl *a* concentration was determined fluorometrically following Parsons et al. (1984). The calibrations from this study were combined with previous ones (C.A. Pilditch, unpublished data) to give a predicted concentration of Chl *a* and total seston within 12% of that estimated from direct water sampling. Biases resulting from

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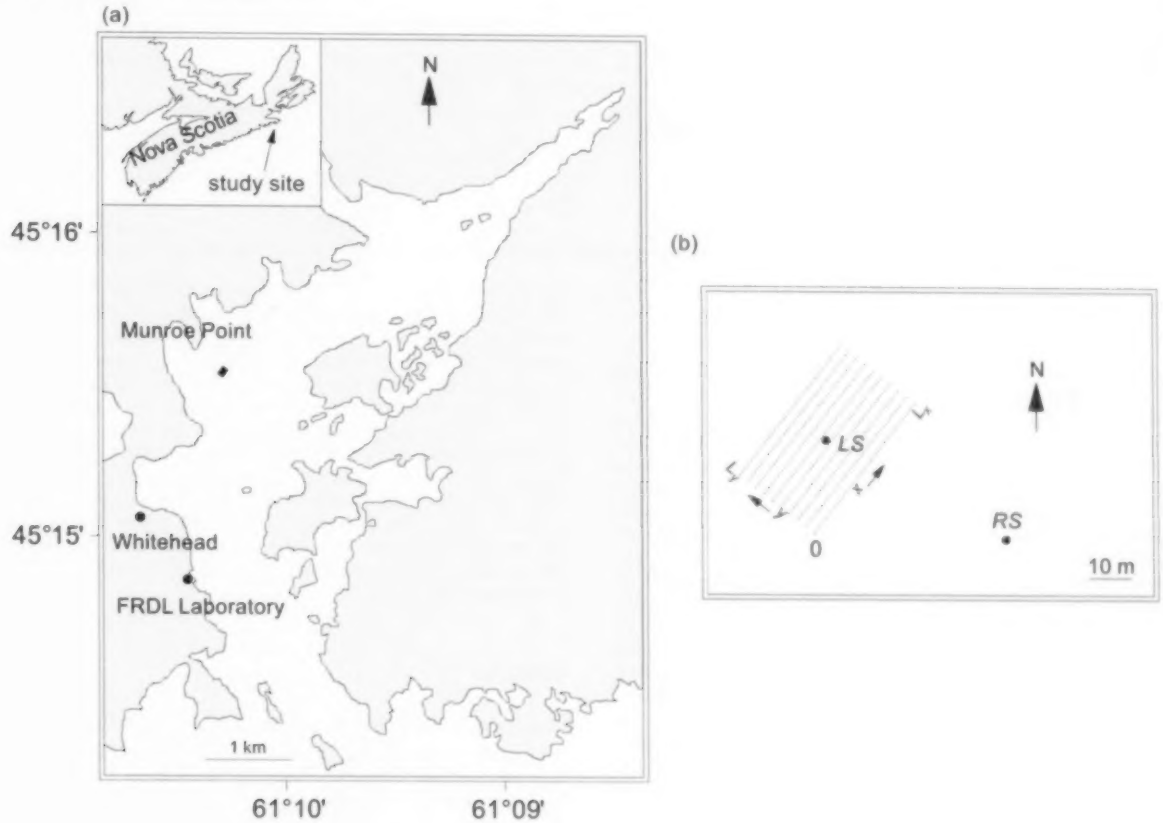
Fig. 1. (*a*) Location of the scallop culture in Whitehaven Harbour, Nova Scotia, Canada. The black box represents the area under cultivation during this study and the broken box the maximum area available for cultivation. (*b*) Details the arrangement of the longlines at Munroe Pt., the coordinate convention used, and the location of the lease site (LS) and reference site (RS) sites. L_x and L_y refer to the lease length and width, respectively.



biotic fouling of optical surfaces on the moored instrument were removed by subtracting an empirical exponential fouling curve determined for this site from the voltage data.

Changes in pigment concentration attributable to primary production and microzooplankton grazing were estimated on two occasions, 7–8 and 9–10 August. Replicated (n = 2) light and dark BOD bottles were filled at 5, 10, and 15 m above bottom and then incubated for 24 h at RS. Net primary production was estimated from the difference between the initial and final dissolved O_2 concentrations (determined with a Orion 97–08 O_2 electrode) in the light bottles assuming a photosynthetic quotient of 1.2 (Thomas 1976).

Seston consumption by scallops in suspended culture was estimated in feeding experiments conducted at the lease processing facility (Fig. 1) between 7 and 10 August. Although water supplied to the laboratory was taken 2 km from the lease, temperature and Chl *a* concentrations were similar (±10%) between the intake and the sampling site. Experiments were conducted in four flow-through chambers (20 cm in diameter × 12 cm, 3.8-L volume), each fitted with a small stirring bar to insure adequate mixing. One chamber was used as a reference chamber and the other three as experimental chambers. Water was delivered to each chamber via Tygon tubing from a constant-volume header tank that maintained flow rates at between 480 and 600 mL·min^{-1}. These flow rates ensured that pigment concentrations were not reduced by more than 30% between the inflow and outflow of each chamber. Scallops collected from the lease (88–95 mm shell height, 9.6–11.4 g dry weight) were acclimated for 1 h during which time they resumed a normal feeding posture (shell gaping and tentacles extended). Several water samples were simultaneously collected from the exit port of each chamber over a recorded time interval to allow calculation of flow rates. Two samples were collected 1 h apart and the chambers were then emptied and the scallops replaced. The feeding rates of 18 scallops were measured over the 4-day period. The concentration of Chl *a* in the outflowing water of an empty reference chamber and three experimental chambers was determined fluorometrically following Parsons et al. (1984). The clearance rate (CR, the volume of water swept clear of Chl *a* per scallop per unit time) was calculated using the formula CR = FR × (Chl a_r – Chl a_s)/Chl a_r where the subscripts "r" and "s" refer to the reference and scallop chamber, respectively (Hildreth and Crisp 1976).

Model approach

The seston concentration (C) within a culture of suspended bivalves is determined by the advection by a mean current ($\vec{U}$) and diffusion (K) of particles from remote sources, the local production of new particles (sources), and the removal of particles due to feeding and sedimentation (sinks). The local time (t) rate of change of seston within a lease can be described by

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Table 1. Variable values used in model runs.

Variable	Value	Source
Scallop clearance rate (CR)	3×10^{-6} m^3·scallop^{-1}·s^{-1}	Field study
Along-lease turbulent diffusion coefficient (K_x)	10 m^2·s^{-1}	Fischer et al. 1979
Across-lease turbulent diffusion coefficient (K_y)	1 m^2·s^{-1}	Fischer et al. 1979
Peak tidal velocity (u_{max})	0.05 m·s^{-1}	Field study
Tidal period (ω)	1.408×10^{-4}·s^{-1}	

	Lease configuration			
	Present (P)	Future 1 (F1)	Future 2 (F2)	Future 3 (F3)
Length (L_x)	80 m	1000 m	1000 m	500 m
Width (L_y)	50 m	250 m	250 m	500 m
Scallop density (M)	12.2·m^{-3}	12.2·m^{-3}	50.4·m^{-3}	50.4·m^{-3}

$$(1) \qquad \frac{\partial C}{\partial t} = -\vec{U}\nabla C + \nabla(K\nabla C) + \text{sources} - \text{sinks}$$

where ∇ is the spatial gradient operator and K the turbulent eddy diffusivity. A number of general assumptions were made to simplify eq. 1. We considered the flow to be one dimensional, a reasonable first approximation given that the dominant flows were oriented parallel to the major axis of the lease and the near homogenous isotropic distribution of Chl *a* in the depth range occupied by the scallops (see Results). Having no field measurements of the spatial variation in flow within the lease or the rate of turbulent diffusion, a uniform flow speed and constant dispersion coefficients were also assumed. At Munroe Pt., scallops were suspended from longlines, but in the model, a homogeneous distribution was assumed. The only source of seston for the scallops was due to the advection and diffusion of particles, and the only sink of seston was due to scallop feeding, which was held constant. Previous studies have shown that the *P. magellanicus* clearance rates are independent of natural seston concentrations between 1 and 15 mg·L^{-1} (Cranford and Gordon 1992; MacDonald and Ward 1994). Field results demonstrated that other sources and sinks of seston (phytoplankton production, zooplankton grazing, sedimentation) within the lease could be considered negligible (see Discussion).

Accordingly, eq. 1 can be simplified to give

$$(2) \qquad \frac{\partial C}{\partial t} = -u_{(t)}\frac{\partial C}{\partial x} + K_x\frac{\partial^2 C}{\partial x^2} - \gamma(C - C_0) - FC$$

where F is feeding coefficient (scallop density (M) × CR) and C_0 is the far-field seston concentration. Although similar equations have been solved for unidirectional flow (Incze et al. 1981), this is inappropriate for Whitehaven Harbour where tidal currents mean that there will be a period of very low flow and the flow will reverse, allowing scallops to refilter water that has already passed through the lease. Therefore, our along-lease flow is parameterized by a sinusoid $u_{(t)} = u_{max}\sin(\omega t)$, where ω is the radian tidal frequency and u_{max} is the maximum tidal velocity. Since we are only modelling the lease in the along-lease direction, we have parameterized across-lease mixing ($K_y\partial^2 C/\partial y^2$) by the term $\gamma(C - C_0)$, where

$$(3) \qquad \gamma = \frac{K_y}{(0.5L_y)^2}$$

and L_y is the width of the lease; γ adds a quasi second dimension to the time-varying flow model, which allows us to consider the lease geometry (L_x relative to L_y) as a factor affecting seston supply.

The seston supply model was solved numerically with a finite difference scheme. An upwind differencing was used for the advective term, and a forward time-centred space scheme was used for diffusion (Press et al. 1990). The model was run from the initial condition of uniform seston concentration (C_0) to a periodic steady state, which was achieved in two tidal cycles. Outside the lease, it was assumed that seston concentration was constant and equal to C_0. The model was parameterized using the field data and results from published studies (Table 1); then the scallop density and lease geometry were varied to simulate the effects of lease expansion on food supply to scallops. The present stocking density at Munroe Pt. is 12.2 scallops·m^{-3}; however, in Sungo Bay, China, 50.4 scallops·m^{-3} are produced in earhanging culture (J. Grant, personal observation; Fang et al. 1996). The higher stocking density in combination with the maximum possible lease size (1000 × 250 m) represents the end member of a range of possible lease expansions at Munroe Pt.

Results

Field measurements of seston supply

Current velocities 10 m above bottom were different inside and outside the lease (Fig. 2). Peak flow speeds at RS reached 18 cm·s^{-1}, whereas inside the lease, speeds above 10 cm·s^{-1} were rare. The mean (±1 SD) flow speed at RS (3.9 ± 2.6 cm·s^{-1}) was 40% higher than that measured at LS (2.3 ± 1.6 cm·s^{-1}). At RS, the flood tide and ebb tide current directions were virtually symmetrical (mean ± 1 SD = 10 ± 38 and 194 ± 44°, respectively) (Fig. 2) and nearly parallel to the long axis of the lease (direction = 20°, Fig. 1), whereas at LS the flood tide direction was slightly more northerly (2 ± 34°) and the ebb tide slightly more westerly (198 ± 47°).

A power spectral decomposition of the current meter records showed that variation in the along-lease (x) velocity was dominated by the semidiurnal tide (12.4-h period) (Fig. 3*a*). The narrow width of the 95% confidence limits at this frequency suggests that the peaks at RS and LS are significant, and furthermore, since they do not overlap, the magnitude of the tidal velocity differs between sites. Other peaks existed at 4–7 days and at 72 min. Since the x velocity component was approximately parallel to the dominant flow direction, power in the across-lease (y) component was less (cf. Fig. 3). The spectral plots show not only the reduced flow rate at LS compared with RS but also that the reduction was frequency dependent. Below 5 cycles·day^{-1}, power at RS was greater than at LS, but above this frequency, power at the two sites was similar (Fig. 3). There was no direction-dependent reduction in flow velocity inside the lease, the x

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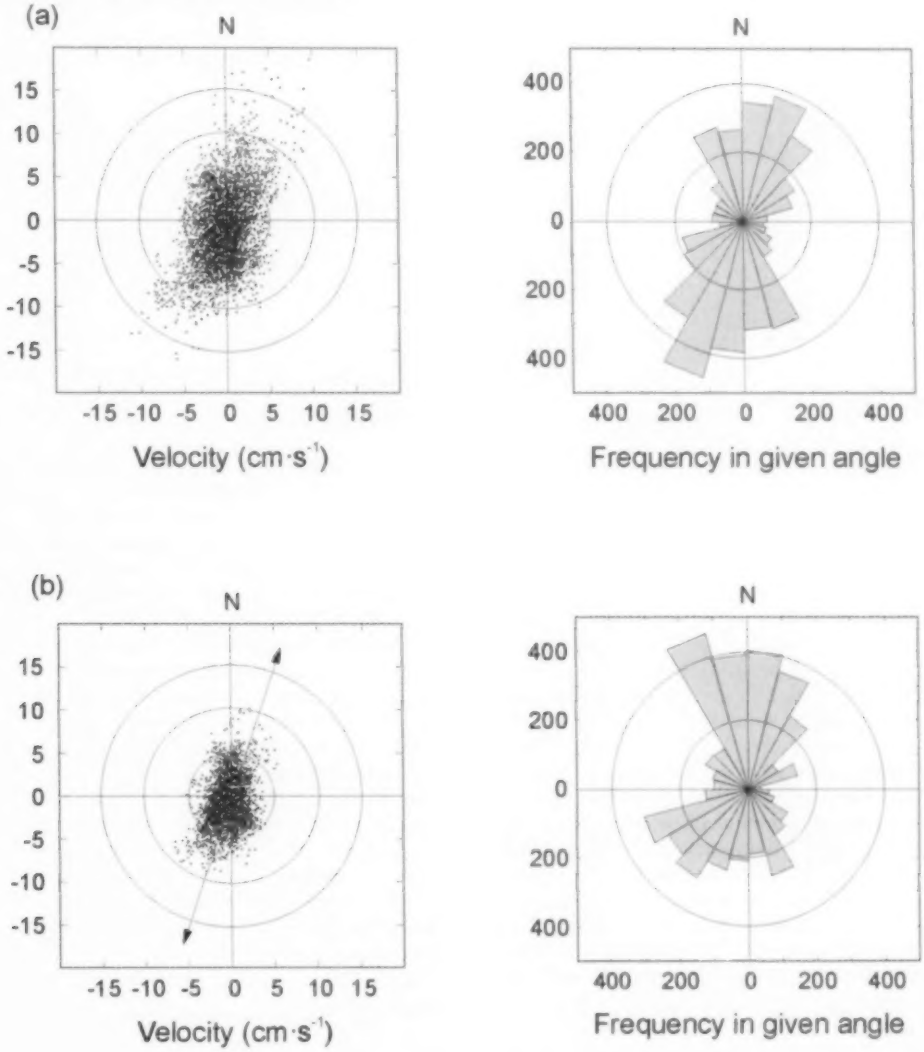
Fig. 2. Polar plots of velocity and frequency direction roses of ambient currents 10 m above bottom at (*a*) RS and (*b*) LS during August 1995. The frequency direction roses represent the counts in 15° intervals. For reference, the along-lease axis (*x* direction) is indicated by the arrow. Directions are relative to true north.



root mean square velocity was reduced by similar amount to *y* root mean square velocity (by 42 and 39% respectively).

During August, Chl *a* concentration entering the lease fluctuated between 0.2 and 4 $\mu g \cdot L^{-1}$, whereas the SPM concentration remained nearly constant between 1.5 and 2 $mg \cdot L^{-1}$ except during a storm when levels reached 4 $mg \cdot L^{-1}$ (Fig. 4*a*). Not surprisingly, spectral decomposition of the SPM record showed no obvious peaks (Fig. 4*b*); however, variations in phytoplankton biomass were dominated by a 6- to 9-d and a 24-h period, with a smaller peak occurring at ~12 h (Fig. 4*c*). Net primary production ranged from 44 mg $C \cdot m^{-3} \cdot day^{-1}$ (15 m above bottom) to 11 mg $C \cdot m^{-3} \cdot day^{-1}$ (5 m above bottom) (mean = 28 mg $C \cdot m^{-3} \cdot day^{-1}$) for incubations begun on 7 August and from 21 to –9 mg $C \cdot m^{-3} \cdot day^{-1}$ for those started on 9 August (mean = 4 mg $C \cdot m^{-3} \cdot day^{-1}$).

Effects of scallop feeding on phytoplankton concentration within the lease

Scallop clearance rates varied between 4.9 and 17.5 $L \cdot h^{-1}$ with a mean (±1 SD) of 9.6 ± 4.7 $L \cdot h^{-1}$ (n = 18), comparable with those measured by Cranford and Gordon (1992) (8–10 $L \cdot h^{-1}$) and MacDonald and Ward (1994) (4–5 $L \cdot h^{-1}$) for *Placopecten magellanicus* of a similar size (8–10 g dry tissue weight) feeding on natural particle assemblages. Clearance rates were measured over a narrow range of ambient Chl *a* concentrations (1.3–1.8 $\mu g \cdot L^{-1}$) and temperatures (14.8–15.3°C), conditions that were very similar to those measured at LS during the two tidal cycles when we looked for evidence of seston depletion. We therefore considered the rates to be representative of scallops feeding in situ.

There was no evidence to suggest that feeding by the scal-

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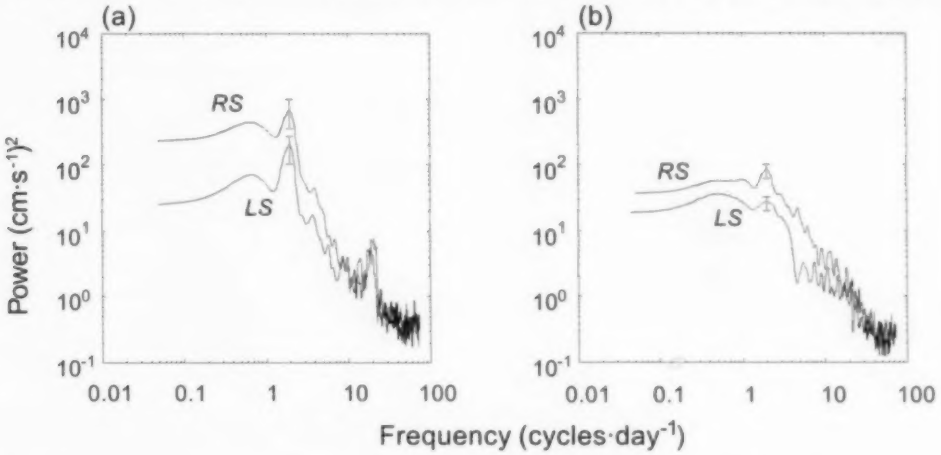
Fig. 3. Variance conserving spectral plots of the (*a*) *x* velocity (along lease) component and (*b*) *y* velocity component (across lease) at RS and LS. The error bars represent 95% confidence limits for the semidiurnal frequency using a 300-point Haning–Bartlett window.



lops reduced Chl *a* concentration within the lease even when flow speeds were low. Chl *a* concentration varied with depth, increasing from 0.8–2 $\mu g \cdot L^{-1}$ between 0 and 5 m and then declining gradually to 1.2 $\mu g \cdot L^{-1}$ near the bed, but differences between RS and LS were not evident. In the depth range occupied by the scallops (5–15 m above bottom), the mean (±1 SD) depth-integrated Chl *a* concentration during two tidal cycles (9–10 August) was 1.72 ± 0.07 $\mu g \cdot L^{-1}$ (n = 22 profiles), the same as that observed at RS (1.72 ± 0.09 $\mu g \cdot L^{-1}$, n = 23). Because the profile schedules for RS and LS were offset by 0.5 h, temporal variations in Chl *a* concentration associated with sampling different water masses may have confounded the detection of scallop feeding. However, when the instantaneous Chl *a* concentration 10 m above bottom at LS was compared with that recorded by the moored fluorometer at RS using linear regression, the slope was not significantly different from 1 (0.95 ± 0.3 (±95% confidence interval), r^2 = 0.68, n = 20). Furthermore, if scallop consumption rates were exceeding the rate of supply by the currents, then a positive relationship between flow speed and Chl *a* concentration within the lease would be expected. Instantaneous flow speed and Chl *a* concentration 10 m above bottom at LS were not correlated (linear regression, r^2 = 0.01, p = 0.68), and even at flow speeds < 1 $cm \cdot s^{-1}$, there was no evidence of reduced Chl *a* concentrations.

Seston supply model

The flow predicted by the tidal model induces a temporal variation in seston concentration within the modelled lease (Fig. 5). As the flow velocity increases during the flood or ebb tide, higher seston concentrations penetrate deeper into the lease. When the flow reverses direction, scallops that were originally in the upstream position are now downstream and therefore feeding on seston-depleted water. Consequently, the maximum variation in seston concentration occurs near the lease edge. At the present lease size and stocking density (Table 1), the maximum predicted seston reduction at the lease midpoint where we sampled for depletion was 5%. The low level of depletion predicted by the model is consistent with the field observations where no detectable effect of scallop feeding could be observed.

The time-averaged seston concentration gives an integrated measure of the food available to the scallops (Fig. 6). Due to the bidirectional flow, the minimum seston concentration occurs in the centre of the lease. Although minimal seston depletion is predicted with the present lease configuration (80 × 50 m) (Table 1), if increased to 1000 × 250 m (F1) (Table 1), then seston concentration in the lease is reduced by as much as 20% and lowered even further if scallop density is increased to 50.4·m^{-3} (F2) (Table 1; Fig. 6). Conserving the area of the F2 lease but altering to dimensions to 500 × 500 m (F3) (Table 1) reduced the predicted depletion from 50 to 30% (Fig. 7). However, the lease geometry that minimizes seston depletion is sensitive to the magnitude of the across-lease exchange term γ. Increasing K_y to 10 $m^2 \cdot s^{-1}$ (equal to K_x and still within the range observed for well-mixed estuaries; Fischer et al. 1979), seston depletion was greater in the rectangular than in the square lease (Fig. 7).

Discussion

Field measurements of seston supply

Variations in current velocity were dominated by three forcing frequencies, of which the semidiurnal tidal component was the most important. Tidal variability in the flow velocity has important implications for the transport of seston to the scallops. During slack water, low flow rates may result in periods of food depletion similar to that observed above epibenthic mussel populations (e.g., Fréchette et al. 1989), and furthermore, the bidirectional nature of the flow could result in water previously depleted of particles reentering the lease. Flow variations with a 4- to 7-day period are most likely due to atmospheric forcing by winds and (or) pressure fluctuations. Higher flows generated by passing weather systems have been correlated with an increase in bay flushing rate on the east coast of Nova Scotia (Heath 1973). The cause of the spectral peak at 8.3 $\times 10^{-4}$ cycles·day^{-1} is unclear. The natural period of oscillation (Pond and

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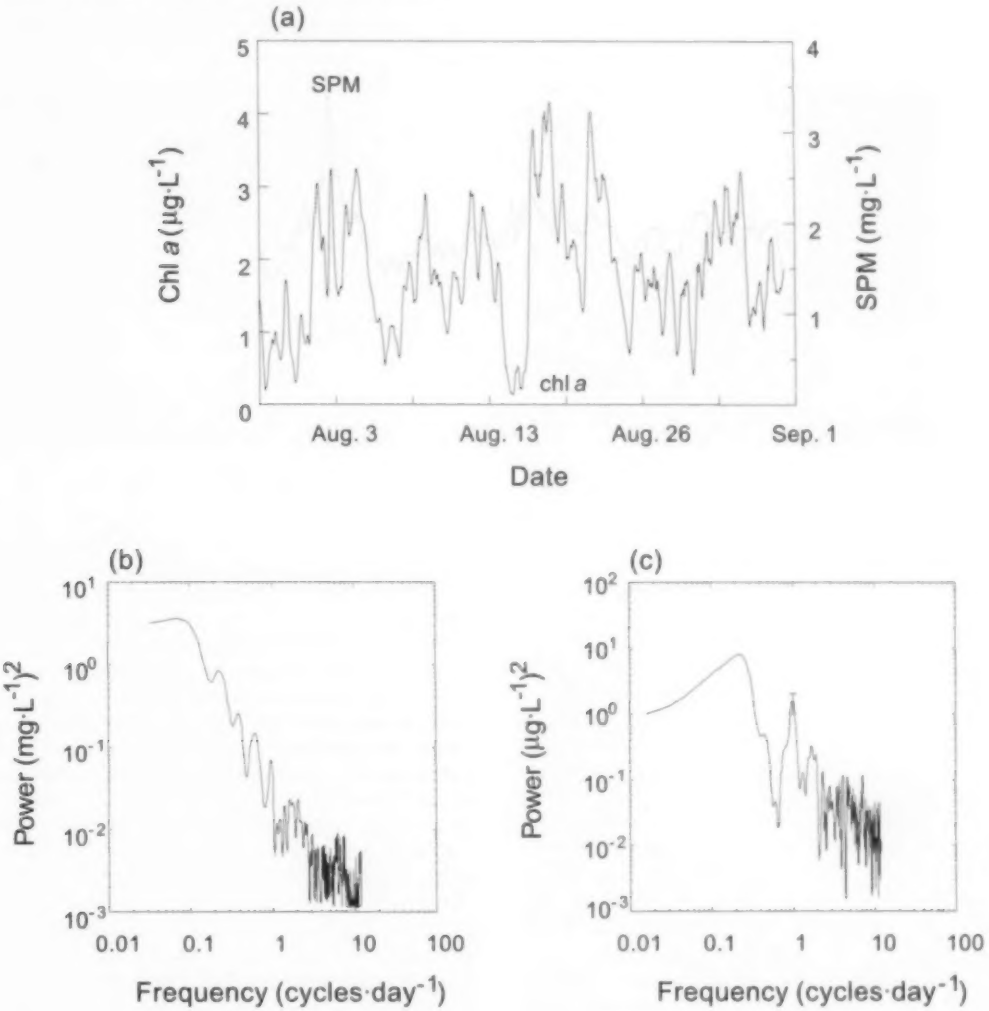
Fig. 4. Variation in the seston concentration 10 m above bottom at RS during August 1995. (*a*) Time series of Chl *a* and SPM concentration derived from calibrated fluorometer and transmissometer voltages (hourly readings have been plotted with a 3-h running mean) and variance-conserving spectral plots of (*b*) SPM and (*c*) Chl *a* concentration. The error bars represent the 95% confidence limits for the diurnal frequency using a 300-point Haning–Bartlett window.



Pickard 1983) for Whitehaven Harbour assuming a mean depth of 18 m and a length of 6 km is ~30 min, less than the observed period of 72 min.

Current speed inside the lease (LS) was lower compared with a reference site 100 m away. Lower flow rates could result from the effects of the suspended culture gear on local flow conditions but may also be related to natural circulation patterns within the bay. The latter is unlikely, however, given that there was no abrupt change in topography in the vicinity of the sites. Furthermore, output from a tidally driven, three-dimensional hydrodynamic model of Whitehaven Harbour (50-m grid; D.R.M. Sturley and A.J. Bowen, Department of Oceanography, Dalhousie University, Halifax, NS B3H 4J1, Canada, unpublished data) indicated that the depth-integrated flow speed at LS during peak flood and ebb tide should be 10% higher than that at RS, the opposite of that observed in these measurements. The predicted flow speeds were 4–5 cm·s^{-1}, somewhat lower than the peak flows observed at RS but in a direction consistent with the current meter measurements. This comparison suggests that the reduction in flow magnitude can be attributed to the effects of the culture gear and not a preexisting spatial difference in the flow pattern.

The lease structure is likely to reduce current speed by a combination of flow divergence and locally increased drag (Grant et al. 1998). Previous studies observed localized accelerations in the flow as it diverged around suspended mussel gear (Gibbs et al. 1991; Boyd and Heasman 1998); correspondingly, there must be a decrease in the flow speed inside the lease to maintain continuity. The reduction in the ambient flow speed inside the lease measured in this study was similar to that cited by Boyd and Heasman (1998) (35–

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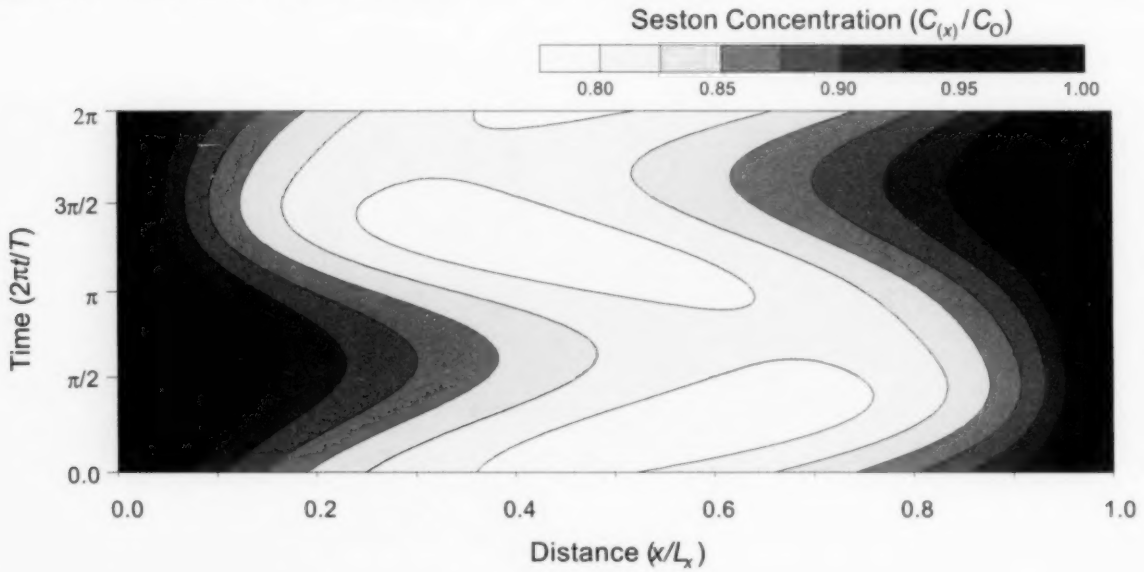
Fig. 5. Predicted temporal variation in seston concentration (relative to the external concentration) within the lease due to a tidal flow as a function of normalized distance. Model inputs are listed in Table 1 together with the F1 lease configuration used to generate the results. Darker shading indicates higher concentrations.



50%) and Gibbs et al. (1991) (70%) within longline cultures of suspended mussels. However, in contrast with the results of Boyd and Heasman (1998), flow direction inside the lease did not align with the major axis of the longlines. On the contrary, flow speeds were attenuated equally in the across-lease and along-lease directions. Direct comparisons between the studies are difficult, since in the previous study the culture unit density was higher and flow velocity was measured with drogues, which may have induced a directional bias. The impact of the culture gear on the flow rate has important implications for the seston supply to scallops, since an increase in the transit time through the lease will allow a greater fraction of the seston to be consumed. This problem could be compounded further with increasing culture unit density (Grant and Bacher 2001).

Despite the 40% reduction in seston flux due to flow attenuation, there was no evidence to suggest that feeding by the scallops reduced seston concentration to a point where the growth of animals downstream was affected. It is unlikely that other sources and sinks of seston (primary production, sedimentation, zooplankton grazing) within the lease may have outweighed any effect of the scallops, making it difficult to detect depletion. Doubling rates for net primary production (0.3–0.06·day^{-1}, assuming a phytoplankton biomass of 1.7 μg·L^{-1} and a carbon to Chl *a* ratio of 40:1 (Parsons et al. (1961)) were 10–50 times lower than the filtration capacity of the scallops (3·day^{-1}). Furthermore, the downward flux of SPM at RS (estimated from sediment traps moored 10 m above bottom from 8 to 11 August; C.A. Pilditch, unpublished data) was 0.3 g·m^{-2}·day^{-1}. With a mean SPM concentration of 2.3 g·m^{-3}, loss due to sedimentation was ~0.13·day^{-1}, again much less than the filtration capacity of the scallops. This suggests that variations in seston concentration, and thus food available to the scallops, were being driven by processes operating at scales larger than the lease.

The large fluctuations in phytoplankton abundance in the 6- to 9-day atmospheric forcing band may be related to increased bay flushing rates. In other Nova Scotian bays, the flushing associated with weather systems in late summer replaces the resident phytoplankton population with cooler (~8°C) coastal water relatively low in chlorophyll but high in nutrients (Platt et al. 1972; Pilditch and Grant 1999). The range of concentrations observed at this forcing frequency is similar to the seasonal variations observed in biweekly samples taken from Whitehaven in April–December (A. Mallet and C. Carver, Department of Fisheries and Oceans, P.O. Box 550, Halifax, NS B3J 2S7, Canada, upublished data). The variations in the seston quality and quantity associated with the water exchange rather than the temperature fluctuations are likely to be an important factor affecting scallop production within the bay (Pilditch and Grant 1999). Vertical migration by phytoplankton may be responsible for the diurnal periodicity in Chl *a* concentration. Water samples collected in early August revealed that the phytoplankton community was dominated by the dinoflagellate *Dinophysis* spp. (50–60%), a genus known to undergo daily migrations (e.g., Kamykowski 1981). This aspect of phytoplankton behaviour means that scallops in suspended culture may experience daily variations in food supply independent of the horizontal advection rates. The ~12-h variation in Chl *a* concentration was related to the direction of flow. The cross-correlation between Chl *a* concentration and flow direction showed a high coherency (0.8) in phase with the ebb tide. The implication is that the upper reaches of the bay have a higher standing stock of phytoplankton, a portion of which is advected through the lease on the ebb tide. This is consistent with biweekly samples of Chl *a*, which showed that the

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Fig. 6. Time-averaged seston concentration (relative to the external concentration) predicted for the P, F1, and F2 lease configuration as a function of normalized distance. Differences between F1 profiles were due to the choice of boundary conditions ($C_0 = C_{(t,L_x)}$ or $C_{(t,L_x+2u_{max}/\omega)}$, see Discussion). Model inputs and the lease configurations used to generate the results are listed in Table 1.

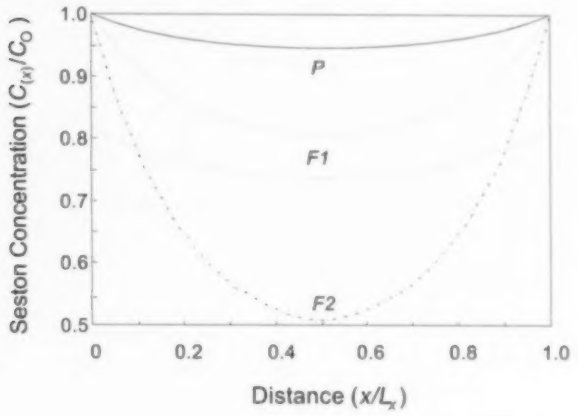
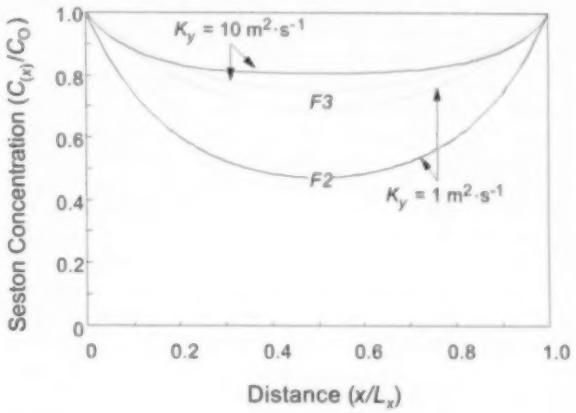


Fig. 7. Model results showing the effect of across-lease exchange and lease geometry on the predicted seston concentrations. The two lease configurations (F2 (solid line) and F3 (broken line)) have the same area but different lengths (Table 1). Increasing the across-lease exchange by an order of magnitude (i.e., increasing K_y from 1 to 10 $m^2 \cdot s^{-1}$) altered the lease geometry, which resulted in the least amount of depletion (see text for details). Model results were time averaged, and seston concentrations are plotted relative to the external concentration as a function of normalized distance. Model inputs are listed in Table 1.



upper part of the bay has higher concentrations than the lease site during August–November (A. Mallet and C. Carver, Department of Fisheries and Oceans, P.O. Box 550, Halifax, NS B3J 2S7, Canada, upublished data). Relocating the lease further up the bay to expose the scallops to higher food levels must, however, be considered in relation to possible changes in the current velocity. A large decrease in flow speed may result in depletion within the lease and outweigh any advantage of higher concentrations entering the lease.

Model predictions

In the present lease configuration, field data and model predictions suggest that even though current speeds were reduced inside the lease, they were still sufficient to ensure an adequate supply of seston. This does not necessarily imply that scallop growth was not food limited, since that will depend on the quality and quantity of the seston relative to internal energy demands (MacDonald and Thompson 1986), but that growth within the lease was not influenced by upstream depletion. The energetic status of the scallops at Munroe Pt. can be estimated by calculating scope for growth (Griffiths and Griffiths 1987). If this is positive, then energy intake exceeds energy expenditure and the excess energy is available for growth, and if this is negative, then the animal is consuming interval reserves and loses weight. At 15°C (mean water temperature during the field study), an adult scallop (10 g dry weight) consumes 117 µmol $O_2 \cdot h^{-1}$ (Pilditch and Grant 1999), or in carbon equivalent units, 1.4 mg $C \cdot h^{-1}$ (assuming a respiratory quotient of 1). Scallop absorption efficiency for coastal seston similar in quality and quantity to that measured at Munroe Pt. is ~70% (Cranford and Hargrave 1994); therefore, 2 mg $C \cdot h^{-1}$ must be ingested to satisfy the metabolic demand. Scallop clearance rates were measured at 9.6 $L \cdot h^{-1}$; thus, seston concentrations <210 µg $C \cdot L^{-1}$ would result in negative scope for growth. The particulate carbon concentration (±1 SD) entering the lease during the field study was 260 ± 50 µg $C \cdot L^{-1}$ (n = 29) (C.A. Pilditch, unpublished data), some 20% higher than that required to satisfy the metabolic demand of the scallops. However, model predictions suggest that expanding the lease may cause seston depletion to exceed 20%, which under the field study conditions would result in negative tissue growth.

Although the model predicted that seston supply at Munroe Pt. would be reduced as lease size and (or) stocking density increased, negatively affecting scope for growth in late summer, it is difficult to extrapolate these results to a temporally integrated estimate of carrying capacity. The problem is that the amount/duration of seston depletion required to affect scallop growth is unknown. To ensure survival, seston supply must meet the basal metabolic demands. Beyond this, further increases in food supply may translate into faster growth rates in the scallops up to some maximum value. Previous authors have arbitrarily set carrying capacity at 50% seston depletion (Rodhouse and Roden 1987; Carver and Mallet 1990), but reductions by as low as 25% significantly lowered the growth rates of the hard-shell clam *Mercenaria mercenaria* (Malinowski and Siddall 1990). Furthermore, the carrying capacity will change with variations in the quality and quantity of seston entering the lease and the internal energy demands of the scallops. For example, the carrying capacity during phytoplankton blooms may be higher than in late summer when food levels are low and respiration rates are high due to gametogenesis (MacDonald and Thompson 1986). The carrying capacity will also decline over the grow-out period, since larger scallops ingest greater quantities of seston. At smaller time scales, scallops

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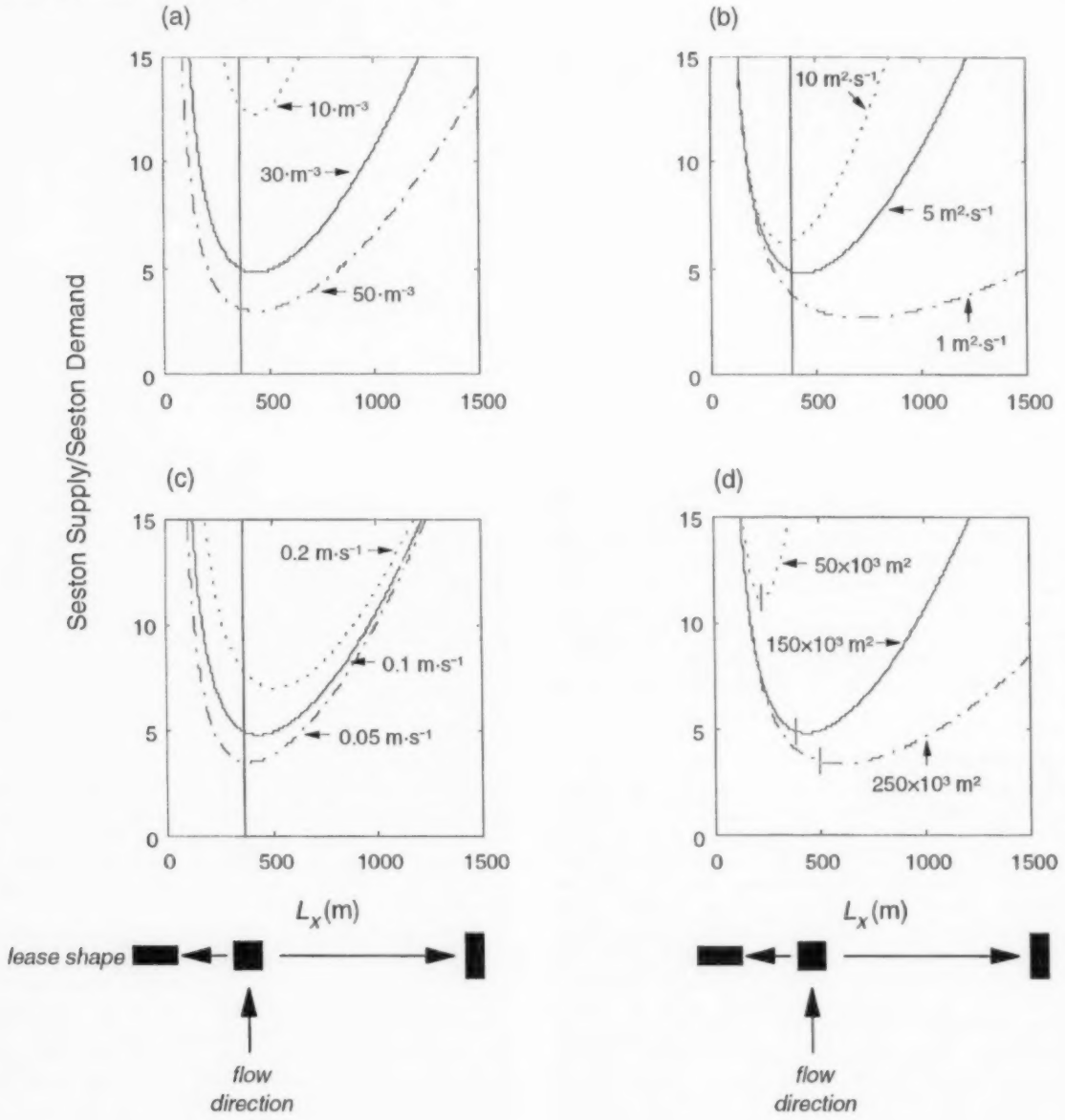
Fig. 8. Plot of nondimensionalized supply terms relative to the feeding term (eq. 4) as a function of lease length (L_x). In each panel, one variable was manipulated, (*a*) scallop density (M), (*b*) across-lease exchange (K_y), (*c*) flow velocity (u), and (*d*) lease area (A), while keeping the remainder constant. For comparative purposes, the solid line in each panel was generated with the same set of variable values: M = 30 scallops·m^{-3}, K_y = 5 m^2·s^{-1}, u = 0.1 m·s^{-1}, A = 150 × 10^3 m^2, and K_x = 10 m^2·s^{-1}. Note that for a fixed A, altering L_x has the effect of modifying the lease geometry. For reference, a vertical line is placed at the value of L_x representing a square lease geometry (i.e., $L_x = L_y$).



may be food limited during portions of the tidal cycle when flows are low. Thus, any estimate of carrying capacity will need to integrate over these different temporal scales, incorporating relationships between metabolic needs and endogenous control over the utilization of internal and external food reserves. Recent simulation models of bivalve energetics (e.g., Grant et al. 1993; Dowd 1997) attempted this, and coupling these models with the current-driven food supply could allow the effect of upstream depletion on growth to be quantified and carrying capacity estimated.

The seston supply models made numerous assumptions in order to simplify complex physical and biological processes. The boundary conditions used to solve eq. 3 have an influence on the predicted seston concentration within the lease.

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In the model, seston concentration at the lease boundaries was set to C_0 and is likely to overestimate the food supply. This boundary condition creates a sharp concentration gradient at the downstream end of the lease, enhancing the diffusive flux of seston back into the lease. In situ, a plume of depleted water will persist downstream of the lease; thus, the concentration gradient at the lease exit will be reduced, decreasing the diffusive flux of seston. Furthermore, when the flow reverses, some of the depleted water is likely to reenter the lease, lowering the seston supply even further. Extending the model domain beyond L_x (so that it is greater than the extent of the lease) permits mixing processes to gradually disperse the depleted plume and for some of it to reenter the lease when the tide changes. Allowing the plume to disperse over a downstream distance equivalent to tidal excursion ($C_{(L_x+2u_{max}/\omega)} = C_0$) decreased the concentration gradient within the lease and the available seston by a further 6–18%. Further increases in the downstream distance did not appreciably alter the predicted seston concentrations within the lease. Therefore, the boundary conditions $C_{(t,L_x)}$ and $C_{(t,L_x+2u/\omega)} = C_0$ represent, respectively, the maximum and minimum range possible of seston concentrations predicted by this model.

While more complicated two- and three-dimensional hydrodynamical models could be generated, their predictive value would be limited without more exact measurements of the dispersion coefficients. With the low advective transport rates, turbulent diffusion was likely to be an important process affecting seston transport at Munroe Pt. Modelling results emphasized that the lease geometry that minimized seston depletion was sensitive to the value of the across-lease diffusion coefficient. We also assumed constant values for the dispersion coefficients, probably an oversimplification given the attenuated flows inside the lease and tidal variations in velocity. In projecting the model to larger leases, the longlines were uninterrupted along the entire length of the lease whereas in reality, the lease would probably consist of a series of smaller blocks. If the dispersion coefficients inside and outside the lease were known, it would be possible to optimize spacing between blocks to allow sufficient mixing of exiting water to offset downstream depletion (Grant 1996). The tidal excursion at Munroe Pt. was 710 m (assuming a peak flow rate of 5 cm·s^{-1}), less than the length of the future lease (1000 m), meaning that a parcel of water could be trapped within the lease for longer than a tidal period. In the model, the exchange of water trapped within the lease was driven by turbulent diffusion, but in situ residual flows within the bay would also contribute. An important consequence of an increased residence time is that the scallops could deplete a greater fraction of the seston and would be more dependent on primary production within the lease than on external sources. When L_x approaches the tidal excursion, a tidally driven flow model may be an inappropriate scale, and future models should focus on the residual bay-scale circulation (Grant and Bacher 2001).

Current meter data suggested that the culture gear reduced flows in the lease, an effect not explicitly accounted for in any of the models. Flow speeds used in model runs were representative of those measured at LS. However, we did not account for any further flow reductions due to increased culture unit density (required for higher stocking levels). Raising the culture unit density is likely to cause a greater flow divergence at the lease entrance and also increased drag within the lease, slowing flows even further (Grant et al. 1998). The reduction in seston flux at higher culture unit densities will be compounded even further by an increase in the filtration capacity. In higher-order models, the effects of the culture gear on the flow rates should be included, since they are likely to play a critical role in the seston supply.

In the model, scallop feeding was described as a first-order process, in that clearance rate remained constant, ingestion varying with seston concentration. Previous studies have shown the clearance rate of *P. magellanicus* to be independent of seston concentration (Cranford and Gordon 1992; MacDonald and Ward 1994) at the levels measured at Munroe Pt. However, when the scallops are exposed to manipulated diets, they are capable of variable feeding behaviour; for example, clearance rates are elevated at low seston concentrations and particle selection occurs as diet quality decreases (Cranford and Gordon 1992; MacDonald and Ward 1994). Although future models should include aspects of feeding behaviour, this is of secondary importance to improving the parameterization of dispersion coefficients.

Generalized relationships between seston supply and demand

Numerical models were used specifically to predict seston supply to scallops at Munroe Pt. and demonstrated that lease size and shape influenced seston supply. Some simple relationships for more general use can be derived from the governing equations in order to judge the potential for seston depletion at other sites. If a unidirectional steady flow is assumed, the point that will be most depleted in seston is at the downstream end of the lease ($x = L_x$). For a bidirectional tidal flow, this point would occur in the centre of the lease. Equation 3 was nondimensionalized by setting the concentration change over L_x equal to C, the velocity equal to u, and then normalising by FC. This scaled the seston input terms (advection, diffusion, and across-lease exchange) to seston removal due to scallop feeding (F) to give

$$(4) \qquad 0 = \frac{u}{L_x F} + \frac{K_x}{L_x^2 F} + \frac{K_y L_y^2}{(0.5A)^2 F} - 1$$

where $A = L_x \times L_y$ (the area of the lease). If the first three terms are much larger than 1, there will be an ample supply of food; conversely, if the first three terms are close to or smaller than 1, there will be seston depletion.

In suspended bivalve cultures, we are interested in determining what lease size and shape optimizes the seston supply for a given set of hydrodynamic conditions. Certain choices of L_x will have the desired effect of increasing the supply terms relative to the feeding term. Maximizing eq. 4 gives a quadratic equation that is difficult to solve. However, plotting the first three terms illustrates the effect of changing L_x, A, K_y, u, and F on the seston supply (Fig. 8). Note that for a fixed A, altering L_x has the effect of modifying the lease geometry; a low value of L_x implies that the lease is wider than it is longer, and vice versa. No maxima in supply relative to feeding exist when L_x is greater than zero, but there is a minimum in all cases. Therefore, there is not a single geometry that will give ideal seston supply given a par-

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ticular combination of A, K_y, u, and F. However, there is a value of L_x that gives a suboptimal geometry for seston supply and should be avoided. In most cases, the lease that approximates a square is not desirable, and a geometry that maximizes either L_x or L_y is better. Increasing or reducing stocking density (F) does not change the location of the minimum but simply increases or reduces the relative importance of supply versus feeding (Fig. 8*a*). Increasing the across-lease exchange term makes long, thin leases more viable than before, reducing the location of the minimum in L_x (Fig. 8*b*). Increasing the flow velocity moves the minimum to a slightly higher L_x, possibly making short, wide leases a better option (Fig. 8*c*). Finally, changing the lease area also changes the position of the minimum (Fig. 8*d*). For smaller leases, the square geometry should be avoided in favour of either a long, thin lease or a short, wide one. For larger leases, a longer, narrower lease results in the poorest seston supply, and in this case, the supply is optimized more readily by a short, wide lease than by a long, narrow one.

The simple relationships shown in Fig. 8 indicate that even if seston is unlikely to be significantly depleted, it is still possible to optimize seston supply to the bivalves at the end of the lease for a given site. For example, if the lease area is restricted to 150×10^3 m^2 with a maximum dimension of 1000 m, L_x can range between 150 and 1000 m. Figure 8*c* suggests that a long, thin lease (L_x = 1000 m) is better in low-flow conditions (0.05 m·s^{-1}) but a short, wide lease (L_x = 150 m) is better in high-flow conditions (0.2 m·s^{-1}). Similar conclusions can be drawn from Figs. 8*a*, 8*b*, and 8*d*. In practise, the choice of lease geometry will be somewhat limited by the size of the bay and other constraints.

In conclusion, at Munroe Pt., scallop feeding had no detectable effect on the food supply, even though particle flux was reduced by 40% due to attenuation of flow by the culture gear. Under present conditions, scallop production was not limited by the upstream depletion of seston but by density-independent factors such as seston quality and quantity. Hydrodynamic processes were, however, important determinants of seston concentrations at scales larger than the lease; tidal mixing processes and bay-scale flushing caused significant variations in phytoplankton concentration which may affect production at the lease scale.

Model simulations suggested that if the area under cultivation at Munroe Pt. was expanded to fill the entire site, seston depletion within the lease could reach 20–50%. The significance of this reduction on scallop production is unknown, yet it is fundamental to estimating carrying capacity. At the scale of the culture unit, studies have demonstrated that competition for food reduces scallop growth (e.g., Parsons and Dadswell 1992; Côté et al. 1994), but the level of food reduction was never quantified. Future studies should therefore examine the effects of known reductions in food supply on scallop production; thus, the levels of seston depletion predicted by the model can be quantified. However, even with such studies, extrapolation in time and space will be difficult. Carrying capacity will vary between sites due to regional differences in primary production and hydrodynamics and also temporally within sites due to seasonal cycles of primary production and scallop internal energy demand. Coupling models of bivalve energetics with seston supply models will provide the necessary temporal integration and could provide an estimate of carrying capacity at the lease scale. A key test of such models will be verification in field studies of the predicted gradients in growth within leases.

The novel part of this study was that the food supply to scallops could be enhanced by altering the lease geometry. The derived relationships still require field testing but suggest that aquaculturists can optimize the shape of the lease to maximize the current-driven food supply. Although these relationships are comparatively simple, they provide a first-order estimate of whether seston depletion is likely to be significant at a given site.

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Designing and evaluating length–frequency surveys for trap fisheries with application to the southern rock lobster

Richard McGarvey and Michael Pennington

Abstract: A survey design for estimating the length distribution of harvested southern rock lobsters (*Jasus edwardsii*) was developed for the South Australian fishery. Experimental sampling was carried out by volunteer fishers in spring 1996 and autumn 1997 to test three proposed survey designs. A variance components analysis indicated that it would be more efficient to sample one pot per trip from all trips rather than the previous design of sampling multiple pots from a few trips. The variation among licenses (fishers) accounts for most of the remaining sample variance. Onboard research sampling by scientists, who in the past measured from all pots on selected trips, was shown to be the least efficient design option in comparison with volunteer sampling by fishers. A sampling protocol where fishers measure one to three pots per trip has been adopted by the South Australian rock lobster fishers. Estimators, based on a three-level sampling hierarchy of pot, day, and license, are presented for estimating the mean and sample variance of the numbers harvested overall and within each length category.

Résumé : Nous avons élaboré un plan d'échantillonnage pour estimer la distribution des longueurs des Langoustes (*Jasus edwardsii*) récoltées dans la zone de pêche du sud de l'Australie. Un échantillonnage expérimental a été mené par des pêcheurs volontaires au printemps 1996 et à l'automne 1997 pour évaluer trois plans d'échantillonnage. Une analyse des composantes de la variance a révélé qu'il était plus efficace d'échantillonner un casier par sortie dans toutes les sorties que d'échantillonner plusieurs casiers de quelques sorties, comme on le faisait antérieurement. Les variations observées entre les détenteurs de permis (pêcheurs) explique presque entièrement le reste de la variance. Un échantillonnage scientifique à bord des bateaux par des chercheurs, qui dans le passé mesuraient les langoustes de tous les casiers lors de sorties choisies, s'est avéré moins efficace que l'échantillonnage volontaire par les pêcheurs. Les pêcheurs de langoustes du sud de l'Australie ont adopté un protocole d'échantillonnage dans lequel ils font des mesures dans un à trois casiers à chaque sortie. Nous présentons des estimateurs basés sur un échantillonnage hiérarchique à trois niveaux, casier, jour et détenteur de permis, pour déterminer la moyenne et la variance de l'échantillon des nombres de langoustes récoltées en totalité et dans chacune des catégories de longueur.

[Traduit par la Rédaction]

Introduction

For exploited populations of species, often invertebrates, that cannot be aged, length samples serve as the principal data source for measuring changes in population structure. Among the range of dynamic stock assessment approaches employing length–frequency samples (Fournier and Doonan 1987; Schnute 1987; Sullivan 1992) are models for continuously growing fish (Deriso and Parma 1988; Fournier et al. 1990) or molluscs (Sainsbury 1982) and for discretely growing crustaceans (Zheng et al. 1998). Several of these models apply specifically to trap fisheries (Bergh and Johnston 1992; Punt and Kennedy 1997). However, to our knowledge, formal estimators for calculating the precisions of trap survey estimates of numbers harvested, or of numbers by length group, have not been published. Length–frequency sampling is usually characterized by high variance because of varying density and the tendency for animals caught together to be more similar than those in the entire population (Pennington and Vølstad 1994). Sample strategies that reduce this variance can, therefore, substantially improve the quality of length-based stock assessments.

In the South Australian southern rock lobster (*Jasus edwardsii*) fishery, a formal survey design was needed for the ongoing monitoring of length structure for yearly stock assessment. As with most trap fisheries, it is not feasible to survey the lobster population directly (e.g., with divers or submersibles); the survey is of the commercial catch.

The aim in this paper is twofold: (*i*) to assess a range of trap survey designs for an optimal choice of survey protocol and (*ii*) to develop estimators of means and their precision (i.e., sample variance), which can be applied to estimate the overall catch and the catch by numbers within each length category. A survey protocol was sought that was optimal in two ways. First, the design should minimize the work and inconvenience required to carry out length measurements in the course of daily fishing operations. To conform as closely

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Table 1. Sampling protocol options for the South Australian rock lobster catch length monitoring survey, 1996–1997.

	Sampling region		
Option	Northern Zone	Northern Southern Zone	Southern Southern Zone
A	5 days, all pots	4 days, all pots	3 days, all pots
B	10 days, 1/5th pots	10 days, 1/7th pots	10 days, 1/10th pots
C	30 days, 1/15th pots	30 days, 1/21th pots	30 days, 1/30th pots

as possible to a sampling protocol that fishers favored, fishers were direct participants in the choice of measurement protocol. Second, for a given number of pots sampled, the estimates of population (i.e., the catch) characteristics should be more precise (yield a lower predicted sample variance) among the range of practical survey designs compared.

The principal outcome is that optimal design is achieved by allocating greater coverage among the highest sampling unit, namely licenses. Thus, in comparing surveys where researchers board vessels (and generally measure all the pots lifted on a given trip-day) with surveys that achieve cooperation from fishers who sample only one to three pots on a subsample of fishing days, the latter strategy achieves far higher precision for equal total numbers of pots sampled.

Materials and methods

The South Australian lobster fishery is divided into two zones, Northern and Southern, managed under effort controls and individual quotas, respectively. A license entitles a single vessel to fish for lobsters using a designated number of traps (pots) varying from about 40 to 80 per vessel. Pots are baited, set individually overnight, and hauled the next morning when captured lobsters are removed and stored live onboard. The season is 7 months in both zones, October–April in the Southern Zone and November–May in the Northern Zone. Effort in both zones is higher at the start of the season, stays high through summer (December–February), and tapers off, more gradually in the Northern Zone than in the Southern where, as quotas are reached, fishing more rapidly ceases. Molting of lobsters occurs in midsummer and peaks in January, which alters the length composition of the population and thus of the catch. Lobsters below the legal size limits of 102 mm carapace length in the Northern Zone and 98.5 mm carapace length in the Southern Zone ("sublegals") must be returned to the sea. Female lobsters carrying eggs, which occur in October–November, are also returned. Most pots do not have escape vents. While some movement occurs, the large majority (88%) of tagged lobsters were recaptured within 5 km of the release site (Prescott et al. 1998)

Catch sampling has been undertaken yearly at sea in both zones since 1991 (Prescott 1992). Fishers are asked to randomly choose specific pots for sampling at the start of each sampling season and mark them by adding a colored tag. On fishing days when sampling is carried out, all the lobsters in any designated sample pot are measured for length and sex, and if female, sexual maturity status is recorded. Empty pots are also recorded. In most years, volunteer fishers did about half of the sampling during the course of commercial fishing operations, with the remainder done by onboard researchers who measured all the pots lifted on a given trip-day. In 1996–1997, the year of data collection for the survey methodology assessment presented here, all samples were carried out by volunteer fishers.

The state lobster fishery waters were divided into three sampling subregions (strata) based on average catch rates (Fig. 1). In the southern Southern Zone, lobsters are densely populated and catch rates in numbers per pot lift are relatively high. In the northern Southern Zone, and to a greater extent in the Northern Zone, mean catches per pot lift are lower. There are two surveys during the 7 months of fishing. The first spans 2 months in southern spring (November–December) and the second is over 3 months in autumn (February–April). January, which is the peak month of molting and hence growth, is not surveyed. To reduce survey cost (measured as volunteer sampling effort by fishers), the first and last months, October and May, during which only one or the other zone is open to fishing, were omitted because these are months with high variation in catches and effort.

Three sampling options were endorsed at a license-holder workshop (Table 1), each giving approximately equal numbers of pots per fisher to sample. Option A was to sample all pots set and lifted on a sample trip (i.e., day) for 3, 4, or 5 sample days. Option C was to sample approximately two, three, or four pots each sample day over 30 boat-days of sampling. Option B was an intermediate strategy of sampling approximately 10 pots per boat-day. To equalize the numbers of lobsters measured by each participating vessel, the sampling protocol took into account the variations in catch rates among the three subregions (Table 1) and differing numbers of pots being set per vessel. Using their preferred sampling option, volunteer fishers sampled 11 665 pots and measured 24 270 lobsters during the 1996–1997 fishing season.

Because traps are subsampled from all those lifted, the days sampled by a fisher are a subsample of days fished, and not all fishers (i.e., licenses or vessels) participate, this survey represents a case of three-stage sampling (Cochran 1977). We denote the primary sampling units as "licenses" (which are equivalent to vessels or boats or individual fishers, skipper, and crew who carry out the sampling work), the second sampling unit as "days" (the days sampled by each fisher out of the full season of fishing days), and the third (lowest) sampling unit as "pots" (i.e., traps). This hierarchy describes surveys of many fisheries using baited traps, notably for crab and lobster.

Selecting a survey design

The number of lobsters, y, in a pot can be written as a function of its deviations from the mean number of lobsters per pot at each sampling stage (Box et al. 1978):

$$(1) \qquad y = \mu + e_l + e_d + e_p$$

where μ is mean number of lobsters per pot lifted in the entire fishery, the e_l component represents the difference between the mean catch by license l and the grand mean μ; e_d represents the difference between the mean catch by license l and its mean catch on day d, and e_p represents the pot-to-pot deviation (given license and day). For the purpose of assessing survey designs, it is assumed that each license fishes the same number of days and sets the same number of pots each trip. If n licenses are chosen at random, m trip-days are chosen randomly for each license, and within each trip, k pots are sampled at random, then the variance of the average number of lobsters per pot ($\bar{\bar{y}}$, which denotes the average number per third-stage unit, i.e., pot) is given by (Cochran 1977)

$$(2) \qquad \mathrm{Var}(\bar{\bar{y}}) = (1 - f_1)\frac{\sigma_L^2}{n} + (1 - f_2)\frac{\sigma_D^2}{nm} + (1 - f_3)\frac{\sigma_P^2}{nmk}$$

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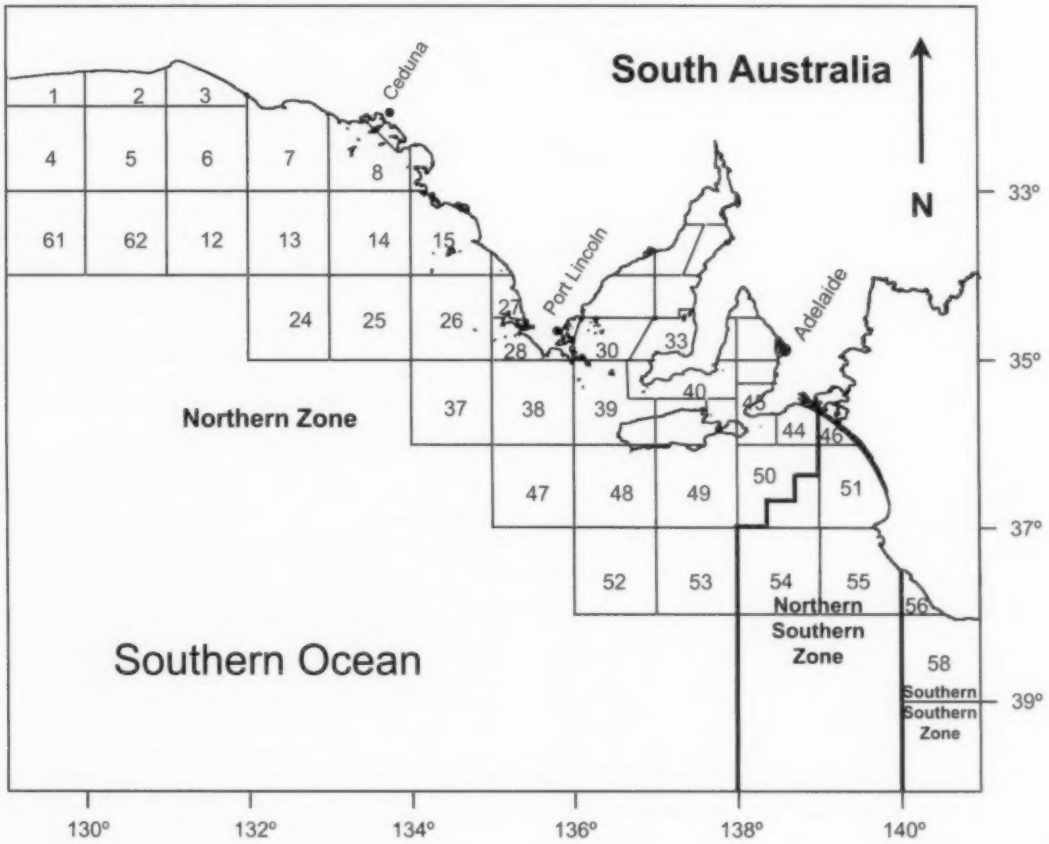
Fig. 1. Map of South Australia showing the three rock lobster survey subregions. The South Australian statistical reporting blocks for catch and effort log data are enumerated.



where σ_L^2, σ_D^2, and σ_P^2 are the variance components for license, day, and pot and f_1, f_2, and f_3 are the proportion of licenses, days, and pots sampled, respectively, at each stage. In most practical situations, the number of days fished and the number of pots set by each license will vary. If a fixed proportion of trips and pots (f_2 and f_3) are sampled for each license, then eq. 2 with m and k replaced by their average values will apply approximately (Cochran 1977).

Equation 2 permits assessment of sampling designs, in particular to determine which gives the lowest sample variance. If all three variance components are nonzero, then sampling more pots per trip (i.e., increasing k) only reduces the last term in eq. 2, leaving the contribution due to variance among days and licenses unaffected. Sampling on more days (increasing m) for each license reduces the variance contributed by the last two components, pots and days. Finally, if the number of licenses sampled were increased (n), then all three sources of variability would be reduced proportionally. Thus, the most efficient design for sampling a fixed total number of pots (= nmk and ignoring the relative costs of using the different sampling schemes) would be to collect pots from as many licenses as possible and then from as many different days fished by each license as possible.

If the lengths of lobsters caught in the same pot or during the same trip or by the same license tend to be more similar than those in the entire catch, then this also implies that sampling should be distributed as widely as possible to more precisely estimate the length distribution of the catch (Pennington and Vølstad 1994).

The length of an individual sampled at random will have an additional fourth variance component due to within-pot variability in length. Lobster length, x, can be expressed as

$$x = \mu + e_l + e_d + e_p + e_w \quad (3)$$

where e_w is the within-pot component. Because every lobster in a sampled pot is measured, the within-pot variance does not contribute directly to the sample variance.

The survey (catch-monitoring) data from 1996 and 1997 are from six strata: spring and autumn surveys in each of the three subregions. The variance component models for these data (eqs. 1 and 3) are completely nested models, and therefore, analysis of variance techniques were used to estimate the variance components for each stratum (Searle et al. 1992). The analysis of variance estimators used are presented in Searle et al. (1992).

To evaluate the efficiency of various sampling schemes for estimating the number of harvested lobsters, the estimates of the variance components for the number of legal lobsters per pot were substituted into eq. 2. The efficiency of a sampling strategy was assessed based on (*i*) the variance of the sample mean, eq. 2, for a given number of pots sampled and (*ii*) the estimated effective sample size, which is the number of pots in a true random sample of the overall catch needed to yield the same precision as that produced by a particular sampling scheme (see Pennington and Vølstad 1994; Folmer and Pennington 2000).

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Table 2. Estimates of the variance components for the number of harvested rock lobsters caught per pot during the 1996–1997 season.

Survey	$\hat{\sigma}^2_T$	$\hat{\sigma}^2_L$	$\hat{\sigma}^2_D$	$\hat{\sigma}^2_P$	n
Northern Zone					
Spring	3.97	0.18	0.52	3.29	3954
Autumn	3.36	0.14	0.42	2.81	1995
Northern Southern Zone					
Spring	5.10	0.41	0.00	4.93	2109
Autumn	3.07	0.53	0.00	2.72	1359
Southern Southern Zone					
Spring	7.01	0.97	0.00	6.25	1463
Autumn	3.04	0.13	0.08	2.89	787

Note: The variance component $\hat{\sigma}^2_T$ is the estimated total variance, $\hat{\sigma}^2_L$ is the license component, $\hat{\sigma}^2_D$ is the trip component, $\hat{\sigma}^2_P$ is the pot component, and n is the total number of pots sampled.

Estimating total catch

The variance components analysis above was used to indicate the sources of variability and to develop a sampling scheme. In this section, estimators for mean and variance are presented. Survey data provide information on the catch per pot and the proportion of the catch in each length bin. The specific quantities to be estimated are the total number of lobsters caught in 4-mm length bins. South Australian rock lobster logbook data provide the total number of days fished and the number of pots set each day by a fisher. Since the number of days fished by each license varies, as does the number of pots set, a ratio-type estimator was used (Cochran 1977).

For license l, an estimate of the number of lobsters caught per pot lift (total, or by length bin) on sample day d (the estimated daily catch per unit effort) is

$$(4.1) \quad \bar{y}_{l,d} = \frac{\sum_{p=1}^{k_{l,d}} y_{l,d,p}}{k_{l,d}}$$

where $k_{l,d}$ is the number of survey pot lifts by license l on sample trip-day d and $y_{l,d,p}$ is the number of lobsters in a particular length category in sample pot p. The estimated average catch per pot lift by license l during the season is given by

$$(4.2) \quad \bar{\bar{y}}_l = \frac{\sum_{d=1}^{m_l} K_{l,d}\bar{y}_{l,d}}{\sum_{d=1}^{m_l} K_{l,d}}$$

where m_l is the number of days that license l collected samples and $K_{l,d}$ is the total number of pots lifted (from logbook records) by license l on sample day d. The estimate of the average catch per pot lift for the fishery is

$$(4.3) \quad \bar{\bar{y}} = \frac{\sum_{l=1}^{n} K_l\bar{\bar{y}}_l}{\sum_{l=1}^{n} K_l}$$

where n is the number of licenses that collected samples and K_l is the total number of pots set (from logbook data) during the season by license l. Finally, the estimate of the total number of harvested lobsters, $\hat{y}_{tot}$, is

$$(4.4) \quad \hat{y}_{tot} = K_{tot}\bar{\bar{y}}$$

where K_{tot} is the total number of pots lifted in the fishery during the season. An approximate estimate of the variance of $\hat{y}_{tot}$ (adapted from Sukhatme and Sukhatme 1970) is given by

$$(5) \quad \mathrm{Var}(\hat{y}_{tot}) = \frac{N^2}{n}(1-f_1)\sum_{l=1}^{n}\frac{K_l^2(\bar{\bar{y}}_l-\bar{\bar{y}})^2}{n-1} + \frac{N}{n}\sum_{l=1}^{n}\frac{K_l^2(1-f_{2,l})s^2_{2,l}}{m_l} + \frac{N}{n}\sum_{l=1}^{n}\frac{K_l^2 f_{2,l}}{m_l^2}\sum_{d=1}^{k_{l,d}}\frac{(K_{l,d}/\bar{K}_l)^2(1-f_{3,l,d})s^2_{3,l,d}}{k_{l,d}}$$

where

$$s^2_{2,l} = \sum_{d=1}^{m_l}\frac{(K_{l,d}/\bar{K}_l)^2(\bar{y}_{l,d}-\bar{\bar{y}}_l)^2}{m_l-1}$$

$$s^2_{3,l,d} = \sum_{p=1}^{k_{l,d}}\frac{(y_{l,d,p}-\bar{y}_{l,d})^2}{k_{l,d}-1}$$

and N is the total number of licenses in the fishery, n is the number of licenses collecting samples, m_l is the number of days that license l sampled, $k_{l,d}$ is the number of pots that license l sampled on day d, f_1 is the proportion of licenses collecting samples, $f_{2,l}$ is the proportion of days fished that license l collected samples, $f_{3,l,d}$ is the proportion of pots sampled by license l on day d, and $\bar{K}_l$ is the average number of pots set by all the licenses in the fishery. An alternative and more flexible technique for estimating the variance is the jackknife method, which in some situations is more accurate than eq. 5 (Cochran 1977; Pennington and Vølstad 1994).

For fisheries where detailed information is available only from the licenses that collect samples, the estimator of the total catch (eq. 4.4) can be modified. For example, if the total number of licenses, N, in the fishery is known but the total number of pots set, K_{tot}, is unknown, then an estimate of the total number caught is given by

$$(6) \quad \hat{y}_{tot} = (N\bar{K}_{l'})\bar{\bar{y}}$$

where $\bar{K}_{l'}$ is the average number of pots set by the sampled licenses. An approximate estimator of the variance of eq. 6 is obtained by substituting $\bar{K}_{l'}$ for $\bar{K}_l$ in eq. 5.

Equations 4.1–4.4 were applied to the six data sets to estimate the total number of lobsters caught by sex and subregion in each 4-mm length-class. Estimates of the standard errors were generated using eq. 5.

Results

Choosing a survey design

Estimates of the three variance components for the number of legal lobsters per pot lift are given in Table 2. Since these data are not balanced, the sums of the variance components are slightly different from the estimated total variances (see Searle et al. 1992). The largest variance component for all the six survey data sets is the pot-to-pot within-day component, σ^2_P. The day-to-day (within-license) component, σ^2_D, is relatively large in the Northern Zone compared with its value in the two Southern Zone subregions. The survey

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Table 3. Assessment of various sampling designs for estimating the number of rock lobsters per pot lift in the spring Northern Zone survey.

Sampling strategy	No. of licenses participated	Days sampled per license	Pots sampled			SE (% of mean)	Effective sample size
			Per day	Per license	Total		
Option A	38	5	60	300	11 400	4.62	827
Option B	38	10	12	120	4 560	4.22	990
Option C	38	30	4	120	4 560	3.77	1243
Plan implemented	38	50	1	50	1 900	4.24	983
Increase no. of licenses sampled	50	50	1	50	2 500	3.33	1594
	60	50	1	50	3 000	2.73	2365
	70	50	1	50	3 500	2.22	3623
	75	50	1	50	3 750	1.96	4602

Note: A total of 75 licenses are registered in the Northern Zone.

license-to-license component, σ_L^2, contributes relatively more to the variance in the Southern Zone.

The estimates of the variance components were substituted into eq. 2 and the resulting formula was used to assess the effect of various sampling schemes on the precision of the estimators of catch. The comparison of sampling designs (Table 3) was based on the variance component estimates for the spring survey in the Northern Zone.

The first three rows of Table 3 summarize the assessment of survey designs based on the three proposed sampling options (Table 1) giving estimated standard errors and effective sample sizes for each. The effective sample size quantifies how well the sampling design performed compared with (not physically realizable) simple random sampling. For example, a simple random sample of 827 pots would yield the same precision obtained by sampling 11 400 pots using option A (Tables 1 and 3). The reason the effective sample size is much smaller than the number of pots sampled is that the numbers of lobsters in pots from the same trip, or from trips made by the same fisher, tend to be more similar than those in the entire population of pots that were set during the season. Of the three sampling options, C was the most efficient, and as expected, it is better to sample from as many days as possible.

For the present level of sampling participation (38 of 75 licenses in the Northern Zone), 50% of the remaining variance (given the sample sizes for pots, days, and licenses as allocated) is due to license-to-license variability, even though the license component only makes up 4.45% of the total variance (Table 2). This source of variance can only be reduced significantly if the number of fishers sampled is increased. One way to encourage more fishers to collect samples is to minimize the number of pots that each needs to sample. If each license sampled one pot per trip for a total of 50 pots during a season (fourth row in Table 3), then the standard error would be 4.24% of the mean compared with 3.77% for option C and each fisher would sample 58% fewer (50 versus 120) pots per season. The last four rows in Table 3 indicate the gain in precision if more licenses sampled one pot per trip. Not only does the precision increase significantly, but the effective sample size becomes greater than the number of pots sampled when the number of fishers collecting samples approaches 100%; if all fishers participate, the survey design is two-stage sampling stratified by license.

The estimated variance components for length (Table 4) yielded similar choice of optimum survey design. As with numbers per pot lift, the license component accounted for a large part of the remaining variance of the estimates. Since all lobsters in a pot are measured, the license component contributed considerably more than it did for numbers to the variance of the estimates, as indicated by the relative size of the remaining components (columns 3–5 in Table 4). Thus, the same conclusion holds for length as for numbers, i.e., for estimating catch at length, it is better to sample as many fishers and then trips as possible.

Table 4. Estimates of the variance components for length of legal-size rock lobsters caught.

Survey	$\hat{\sigma}_T^2$	$\hat{\sigma}_L^2$	$\hat{\sigma}_D^2$	$\hat{\sigma}_P^2$	$\hat{\sigma}_W^2$	n
Northern Zone						
Spring	239.3	20.4	16.9	16.6	186.1	5689
Autumn	381.3	29.4	36.1	19.6	298.8	2551
Northern Southern Zone						
Spring	211.7	44.0	11.3	12.5	146.8	3228
Autumn	475.0	63.2	24.3	66.5	325.6	1510
Southern Southern Zone						
Spring	100.7	8.7	0.0	10.9	82.8	3226
Autumn	237.2	18.1	0.0	54.5	187.0	1003

Note: The variance component $\hat{\sigma}_T^2$ is the estimated total variance, $\hat{\sigma}_L^2$ is the license component, $\hat{\sigma}_D^2$ is the trip component, $\hat{\sigma}_P^2$ is the pot component, $\hat{\sigma}_W^2$ is the within-pot component, and n is the total number of lobsters measured.

Estimation of total numbers harvested by length-class

Equations 4.1–4.4 were used to estimate the number of lobsters caught in each 4-mm length bin by sex for spring and autumn in the three subregions, generating frequency distributions for the 12 data sets (Fig. 2). The error bounds of the estimated numbers (Fig. 2) were calculated as the square root of the variance from eq. 5. It should be noted that the estimates for legal-size lobsters are harvest estimates, while those for sublegals and egg-bearing females are estimates of the numbers caught and subsequently returned to the sea. The different nature of the estimates, legals sampled without and sublegals with replacement, needs to be considered when assessing or modeling the stock.

Discussion

Conveniently, the strategy yielding lowest variance was the one that fishers preferred. Survey polls by mail, a series

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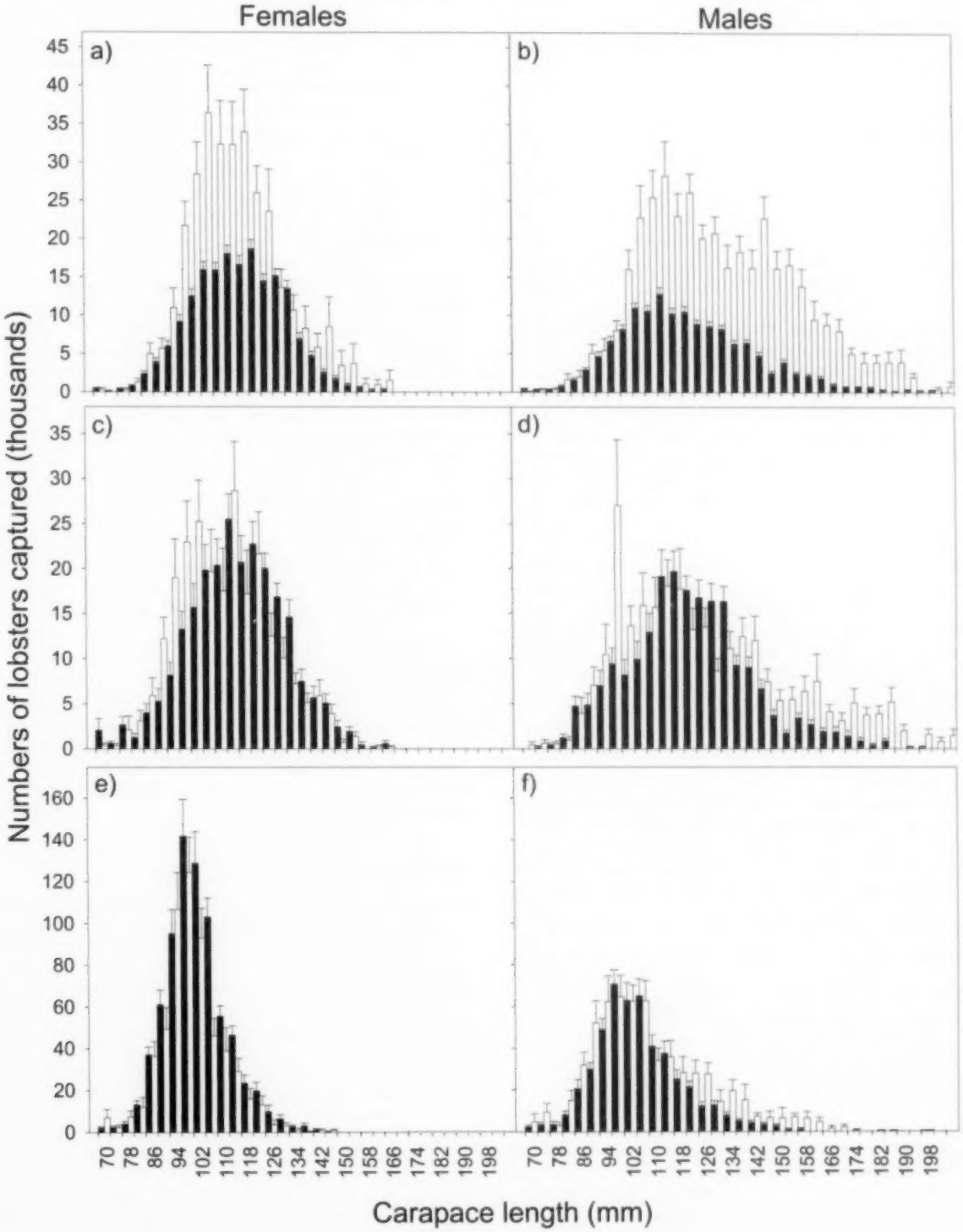
Fig. 2. Estimated length–frequencies of the total numbers of rock lobsters caught, by sex, survey region, and season (spring = black; autumn = white), in 4-mm bins. Error bars indicate 1 SE (from eq. 5) of estimated number caught in each length bin. (*a* and *b*) Northern Zone; (*c* and *d*) northern Southern Zone; (*e* and *f*) southern Southern Zone.



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of port meetings with fishers, and a final large 1-day workshop with all scientists and most participating volunteer survey fishers indicated clear preference for the sampling protocol of fewer pots on correspondingly more days.

For a fixed number of pots, sampling fewer pots per trip and increasing the number of trips sampled will increase the effective sample size for the number of pots sampled and for the number of individual lobsters measured (Pennington and Vølstad 1994). The problem of obtaining a small effective sample size compared with the number of animals actually measured appears to be much less severe with trap surveys than with trawl surveys that have been examined (Pennington and Vølstad 1994; Folmer and Pennington 2000). This is because a pot lift is a relatively small sample unit compared with the haul of a net, and small sampling units are generally more efficient than large units for surveying marine populations (Pennington and Vølstad 1991, 1994; Gunderson 1993).

Vessels (i.e., licenses) that did not participate in the volunteer survey are surveyed by onboard researchers who measure all lobsters brought up in pots during a sample trip-day (in general, chosen opportunistically). The results above, indicating that sampling all pots for a small number of days is the least efficient sampling regime, imply that researcher effort is being employed inefficiently, and thus, sampling entire trips by researchers has now been significantly curtailed.

The most efficient way to increase the precision of the southern rock lobster catch surveys is to encourage more fishers to participate as volunteers. This reduces sample variance (and possible biases) by gathering a more representative sample. Additional fishers may participate if the amount of work involved in sampling is reduced. Towards this end, the new sampling protocol is for a fisher to sample one pot from each trip. Among the survey designs analyzed, this design will generate the most precise estimates for a given number of sampled pots, is the one that the fishers prefer, and greatly reduces the number of pots sampled by each volunteer fisher during a survey. Some fishers nevertheless chose to continue sampling two or three pots as they had done previously.

The choice of order for the three-level sampling hierarchy follows the actual procedure by which sample units were selected in practice. Thus, while in theory there is no reason a sample protocol could not be constructed where days were the highest level of sampling and licenses were chosen from each randomly chosen sample day, in the South Australian rock lobster fishery, this protocol was not followed. In general, there will always be some licenses that do not participate, so the first level of selection, for that reason, is of those licenses that do participate in sampling. If, however, a sampling protocol were adopted in another fishery where days were selected first, then the same equations presented above for the sample means and variances would continue to apply, although inputs and outputs to each level would be reassigned accordingly.

The difference in the classification of sampling, without and with replacement, for the two cases of (*i*) legal lobsters that are kept after being measured and (*ii*) sublegals and egg-bearing females that are returned to the sea, poses no problem in practice, since the percentage of the population actually returned to the sea in either the spring or the autumn surveys is small, being less than 1%, so very few are actually resampled a second time. Moreover, the use of this survey information for legals and sublegals differs. Legals are used to characterize the catch in stock assessment estimation modeling. Sublegals are taken as a time series of "prerecruits," assuming that in the following year, most will enter the legal stock. If exploitation rates are higher than in South Australia so that a significant number of sublegals are resampled, this should be taken into account when estimating an abundance index or a length distribution for the sublegals.

In qualitative analysis of a length survey for the same species of lobster in New Zealand (Starr and Vignaux 1997), no strong differences were observed between researcher- and volunteer-fisher-gathered survey numbers per pot lift and in the corresponding length–frequency distributions. However, assessment was subjective scoring by eye of the graphed length distributions, and for a considerable number of samples (by month and statistical area, summed over 3 years of comparisons), the two surveys failed the subjective equivalence test, underlying a need for more formal methods to quantify survey performance and to calculate the sample variances.

Improved length-based stock assessments would be achieved by explicit recognition of calculated confidence intervals of survey catches by length. In stock assessment models, a weighting on each fitted length bin frequency could be assigned inversely proportional to the calculated sample variance using eq. 5 or its equivalent (Seber 1973). And it appears that more efficient designs are achieved by use of volunteer-fisher sampling, which permits more widely representative samples and thus lower sample variances.

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Dynamics of individual growth in a recovering population of lake trout (*Salvelinus namaycush*)

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Abstract: In 1976, the Wisconsin Department of Natural Resources established a refuge for a nearly depleted population of lake trout (*Salvelinus namaycush*) at Gull Island Shoal, Lake Superior. The refuge was intended to reduce fishing mortality by protecting adult lake trout. We examined the growth dynamics of these lake trout during the period of recovery by comparing estimates of individual growth before and after the refuge was established. Our estimates are based on an annual mark–recapture survey conducted at the spawning area since 1969. We developed a model that allowed mean growth rates to differ among individuals of different sizes and that accommodated variation in growth rates of individuals of the same size. Likelihood ratio tests were used to determine if the mean growth increments of lake trout changed after the refuge was established. Our results suggest that growth of mature lake trout (particularly wild fish) decreased significantly in the postrefuge period. This decreased growth may have been associated with a reduction in food availability. We also observed reductions in growth as wild fish grew older and larger, which suggests that the growth of these fish may be adequately approximated by a von Bertalanffy growth model if it becomes possible to obtain accurate ages.

Résumé : En 1976, le Ministère des richesses naturelles du Wisconsin a établi un refuge pour une population surexploitée de Touladis (*Salvelinus namaycush*) sur le haut-fond de Gull Island dans le lac Supérieur. Il s'agissait de réduire la mortalité due à la pêche en protégeant les Touladis adultes. Nous avons suivi la dynamique de la croissance de ces Touladis durant la période de récupération en comparant les estimations des croissances individuelles avant et après l'établissement du refuge. Nos estimations proviennent d'inventaires de marquage et de recapture réalisés sur les frayères chaque année depuis 1969. Nous avons élaboré un modèle incorporant des différences de taux de croissance moyenne chez des individus de tailles différentes, de même que des variations dans les taux de croissance d'individus de même taille. Des rapports de vraisemblance ont permis de vérifier si les accroissements moyens des Touladis avaient changé après l'établissement du refuge. Nos résultats laissent croire que la croissance des Touladis à maturité (particulièrement celle des poissons sauvages) a décru de façon significative depuis l'existence du refuge. Ce déclin dans la croissance est peut-être associé à une baisse de disponibilité de la nourriture. Nous avons aussi observé une diminution de la croissance à mesure que les poissons sauvages vieillissaient et grossissaient; il apparaît donc que le modèle de croissance de von Bertalanffy puisse décrire de façon adéquate la croissance de ces poissons, à la condition que l'on sache déterminer leur âge avec précision.

[Traduit par la Rédaction]

Introduction

Rehabilitation of depleted fish stocks has received widespread attention and motivated research on fish population and community dynamics in the Laurentian Great Lakes. At the species level, rehabilitation typically involves reduction of mortality rates to permit populations to increase in size and ultimately to be self-sustaining. Rehabilitation goals for fish species may sometimes aim to increase population size to support commercial or sport fisheries of a certain magnitude. Rehabilitation goals for the lake trout (*Salvelinus namaycush*), a native fish in the Great Lakes, include such objectives (Hansen 1996).

In the last century, lake trout populations throughout the Great Lakes have undergone extreme fluctuations in abundance, due in part to overfishing and sea lamprey (*Petromyzon marinus*) predation (e.g., Hansen et al. 1995). Such changes resulted in economic loss to the commercial fishery, decreases in genetic diversity, and loss of stocks and generally contributed to unstable trophic conditions in the lakes. One of the few lake trout populations in the Great Lakes to withstand such intense mortality rates was the Gull Island Shoal population in southwestern Lake Superior. Lake trout abundance at Gull Island Shoal was at an all-time low in the early 1960s (Swanson and Swedberg 1980), but the population responded positively to aggressive fishery management measures that increased stock size and reduced mortality rates. Since the early 1960s, stock density of lake

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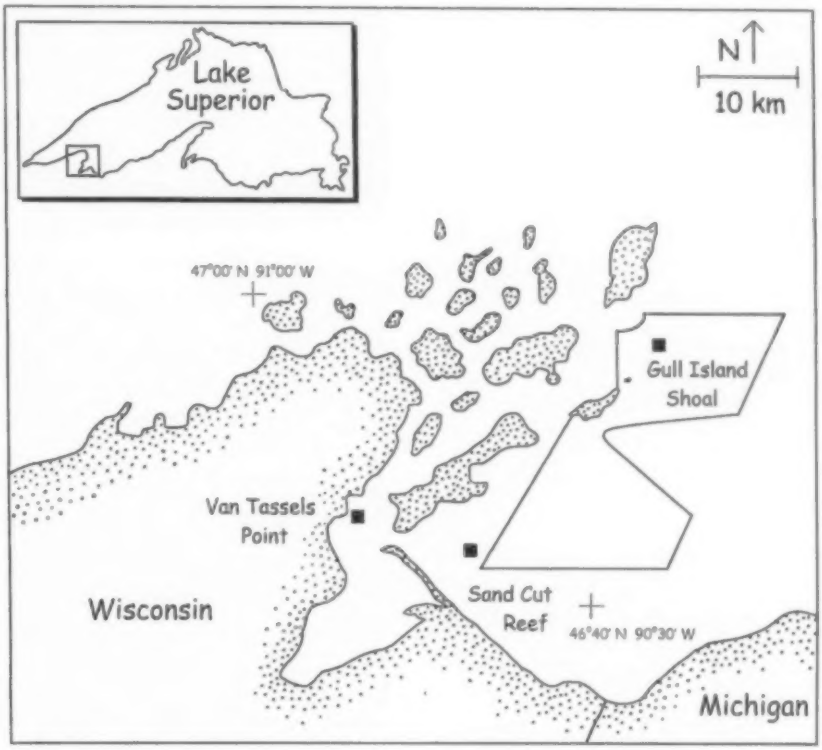
Fig. 1. Study site showing the location of the 70 000-ha refuge (bordered by the solid line) and gillnet sampling sites (squares) in the Gull Island Shoal area of Lake Superior.



trout in Lake Superior was increased through supplemental stocking of hatchery-reared fish, and mortality rates were reduced by controlling sea lamprey predation, placing harvest and effort restrictions on commercial and recreational fisheries, and creating two refuges in Wisconsin waters (Hansen et al. 1995).

The Gull Island Shoal refuge, which encompasses a 70 000-ha area near Gull and Michigan islands (Fig. 1), was established in early 1976 in response to increasing fishing pressure from sport and commercial fishers (Schram et al. 1995). Fishing within the refuge was prohibited. Changes in fishing mortality rates due to the refuge were expected to affect the lake trout population at Gull Island Shoal because many mature fish stay within 40 km of the refuge and return to the same area to spawn (Rahrer 1968). Marked hatchery-reared fish that were observed up to 9 years at liberty exhibited similar movement patterns and tendencies (Pycha et al. 1965).

At Gull Island Shoal, the ratio of wild to hatchery fish changed dramatically through time. In the late 1960s, hatchery fish composed a significant portion of the population (Swanson and Swedberg 1980; Hansen et al. 1995), whereas now most of the population is comprised of wild fish (Hansen et al. 1995). Throughout Lake Superior, growth rates of hatchery and wild lake trout were found to differ (Hansen 1994; Negus 1995). If these differences are significant, temporal changes in the proportion of hatchery-raised lake trout may confound any attempt to assess changes in growth over time. To be conservative, we assess the dynamics of growth of these two subpopulations of lake trout separately.

Here, we investigate the effects of the Gull Island Shoal refuge on growth rates of lake trout by comparing estimates of growth before the refuge was established with estimates of growth during the period of recovery (i.e., after the refuge was established). We examine the growth dynamics of hatchery and wild fish separately to avoid the potentially confounding effects of changes in stock composition. Our estimates of growth are based on mark–recapture studies of lake trout conducted annually since 1969 at Gull Island Shoal. These lake trout include wild and hatchery fish but may be considered members of a unit stock because we examined only individuals that returned to the spawning site.

Materials and methods

Mark–recapture

A detailed description of the study area (Gull Island Shoal, Lake Superior) and the mark–recapture survey are given in Schram et al. (1995) and Fabrizio et al. (1996). Briefly, lake trout were captured annually with gill nets set on the spawning reef during the spawning season (October–November) from 1969 to 1996 (Fig. 1). The graded-mesh gill nets (114- to 178-mm stretch mesh, either nylon or monofilament), which were deployed by personnel from the Wisconsin Department of Natural Resources, were intended to capture primarily large mature lake trout. Live fish in good condition were measured and tagged with individually numbered anchor tags (Floy tag FD-67, FD-67C, FD-68BC, or FD-94). We used the presence or absence of fin clips to indicate origin of the fish: hatchery

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Table 1. Number of lake trout tagged and released annually in the Gull Island Shoal area of Lake Superior.

	No. released		No. recaptured at least once following the year of release	
Year released	Hatchery	Wild	Hatchery	Wild
Prerefuge				
1969	478	232	22	39
1970	1 189	369	18	30
1971	709	548	16	46
1972	842	1 454	22	45
1973	420	692	21	49
1974	633	1 578	17	63
Total	4 271	4 873	116	272
Postrefuge				
1975	291	583	19	105
1976	412	1 143	20	208
1977	541	1 775	20	311
1978	484	2 412	31	435
1979	653	2 060	36	384
1980	294	2 124	26	390
1981	361	1 957	31	422
1982	397	1 913	18	281
1983	256	2 525	12	295
1984	513	1 918	46	223
1985	402	2 098	22	171
1986	355	1 747	17	166
1987	247	1 457	19	173
1988	252	2 538	12	293
1989	339	2 586	29	342
1990	201	1 951	14	189
1991	220	2 075	13	168
1992	229	2 088	18	181
1993	236	2 150	10	107
1994	199	1 888	4	54
1995	85	1 390	1	25
Total	6 967	40 378	418	4923
Grand total	11 238	45 251	534	5195

Note: For the prerefuge period, the number of fish that were tagged and recaptured at least once excludes those that were recaptured after 1975.

fish were fin clipped before stocking in Lake Superior. All recaptures were monitored by Wisconsin Department of Natural Resources personnel; recaptured fish in good condition were rereleased after retagging. We did not use tag-return information from tribal, commercial, or sport fishers because of uncertainties in reporting (e.g., date of capture, tag number, length measurement) and unknown reporting rates.

In addition to the presence of fin clips, the mark–recapture data included information about dates of initial capture and recapture, initial fish length, fish length at each recapture, and origin of fish (wild or hatchery). Data were examined for the presence of outliers, and observations containing recording or data entry errors that could not be corrected by consulting field sheets were omitted from further consideration. However, fish that exhibited unusual growth, including apparent shrinkage, were retained in our statistical analysis, as their validity could not be ruled out objectively (apparent shrinkage of at least 1 cm was evident in only 1.9% of all observations).

Of the 56 489 lake trout tagged in our sampling efforts, 5729 fish were recaptured at Gull Island Shoal at least once (Table 1). Growth of lake trout during the prerefuge period was estimated from observations on 388 fish tagged between 1969 and 1974 and recaptured between 1970 and 1975. Although some of the fish tagged in 1969–1974 were recaptured after 1975, those recapture observations were not considered in our analysis because growth of fish after 1975 was assumed to reflect postrefuge conditions. Growth of lake trout during the postrefuge period was estimated from 5341 fish tagged in 1975–1995 and recaptured between 1976 and 1996. Some of the fish in our study (pre- and post-refuge periods) were first captured and recaptured in the same year (i.e., time at liberty <1 month). In our analysis, we considered the average of these two observations (which usually differed by <1 cm) as the size at first capture.

Statistical analysis

Growth rates of fish are commonly estimated by specifying the conditional mean size of an individual as a function of its age or, in the case of marked populations, its time at liberty. In a well-known example of this approach (the von Bertalanffy (VB) growth model), two parameters (the Brody coefficient k and the maximum size L_∞) specify a progressive reduction in growth rate as fish grow older and larger (Fabens 1965).

The VB model is useful in many settings, but it is unlikely to provide an adequate approximation of the sizes of lake trout observed in our study. Our sampling methods were devised to capture primarily large, mature fishes and are not representative of all ages of lake trout in the population. Consequently, estimates of k and L_∞ cannot be interpreted as population averages (applicable to all ages of fish) without potentially falling prey to errors of extrapolation, which are well known in this setting (Knight 1968). Furthermore, the information we have on the growth of each individual is limited to a few observations (typically one to three recaptures per fish), and we are concerned that these would be inadequate for computing accurate estimates of the parameters of a VB model modified to include variation in growth of individual fishes (Sainsbury 1980). Our concerns are based, in part, on the mixed success of published analyses of growth data simulated under assumed levels of variation (and correlation) in k and L_∞ among individuals of a marked population (Hampton 1991; Palmer et al. 1991). When the size of an individual is observed only a few times during its lifetime, these studies, as well as our own simulation work, have revealed various difficulties in computing accurate and reliable estimates of VB growth parameters. For example, Hampton (1991) and Palmer et al. (1991) simulated the body sizes of a population of marked animals at the time of release and at the time of a single recapture using assumed average values of k and L_∞ and assumed levels of individual variation (and covariation) in these parameters. Palmer et al. (1991) estimated k and L_∞ for a single set of simulated data (see their table 6). Their estimates were actually further from the true parameter values than estimates based on Fabens' (1965) method, which can produce biased estimates even in large samples (James 1991). Although Palmer et al. (1991) noted the disparity in their estimates, neither the relative frequency of their unsatisfactory results nor the average performance of their estimator can be assessed without conducting additional simulations (and analyses) of data. In a similar but more thorough study, Hampton (1991) showed that average values of k and L_∞ could be estimated reasonably well but levels of individual variation in these parameters were biased and imprecise (see Hampton's tables 4 and 5).

Twenty years have elapsed since Sainsbury (1980) demonstrated the dramatic effects that individual variation in growth can have on estimates of average VB growth parameters, yet few investigators have responded to his recommendation that "a topic urgently in need of examination is the form of the joint distribution of k and L_∞ in animal populations." Several methods have been developed for estimating average VB parameters when maximum size (L_∞), but not k, is assumed to vary among individuals in a population (e.g., James 1991; Wang et al. 1995). Sainsbury (1980) noted that this

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assumption seemed reasonable for many fish species but in other species of fish and in other animals, variation in k was also important (e.g., see Xiao 1994). Furthermore, it is not difficult to imagine growth histories that imply a positive correlation between k and L_∞ (e.g., suppose animals that grow faster early in life also tend to achieve larger maximum sizes). In the absence of prior information about differences in growth of individuals, we argue (as did Sainsbury 1980) that a VB model should include parameters for individual variation (and covariation) in both k and L_∞. Fitting the sizes of recaptured animals to less complex VB models can yield biased or overly precise estimates of a population's average growth curve.

Estimation of individual growth increments

As an alternative to models based on the VB growth function, we estimate growth of lake trout using a relatively simple approach that allows the mean growth rate to differ among animals of different sizes. Our approach also accommodates variation in growth among individuals of the same size. The lake trout encountered in our capture–recapture survey were observed at approximately the same time each year; therefore, we develop the following model of the observable sizes of each fish in the year of capture and in each subsequent year when a fish may have been recaptured.

Let Y_j denote a random variable for the size of a fish after j years at liberty. The growth history of an individual fish is represented in the sequence $Y_0, Y_1, \ldots, Y_p$, which we summarize by the vector Y. In our capture–recapture samples, an observed value of Y, say y, was generally incomplete because each fish may have been seen only a few times during its p years at large. However, since the size at first capture was measured for every fish, we can represent the observable sizes of any individual as $y = (y_{obs}, y_{mis})'$, where y_{obs} corresponds to the sizes that were actually observed on one or more sampling occasions and y_{mis} corresponds to the sizes that were missing (i.e., unobserved) because the fish was not recaptured in those years.

We estimate growth increments of individuals by modeling the observable growth histories of lake trout whose sizes at first capture were approximately equal. The discretization of sizes at first capture into categories is necessary because the incremental growth of an individual during its time at liberty may depend, in part, on its initial size. We assume that the observable sizes of these lake trout in each of their p years at liberty are normally distributed: $Y \sim N(\mu, \Sigma)$, where μ is a $(p + 1)$-dimensional vector of mean sizes and Σ is a $(p + 1) \times (p + 1)$ matrix of variances and covariances. We deliberately place no restrictions on μ or Σ so that the pattern of growth is fully determined by the data, not by a functional relationship between each element of μ and its corresponding time at liberty (i.e., the VB approach). We also leave the form of Σ unspecified, which allows the variation in growth among fishes to change with time at large instead of remaining constant. The latter restriction is common in models of repeated measures that assume compound symmetry for Σ (Searle et al. 1992).

We define a p-dimensional vector of individual growth increments, Q, in terms of the parameters of our model:

$$Q = (\mu_1 - \mu_0, \ldots, \mu_p - \mu_0)'.$$

To estimate Q (and Σ of course), we assume that the observable growth histories of different fishes are independent and that the probability of observing an individual during each attempted recapture does not depend on the unobserved sizes in y_{mis}. The latter condition, which is equivalent to the missing-at-random assumption commonly entertained in analyses of incomplete data (Little and Rubin 1987; Schafer 1997), ensures that the mechanisms responsible for creating y_{mis} may be safely ignored (i.e., not explicitly modeled) without compromising estimates or inferences about the model parameters (μ and Σ) or Q.

Let (Y_{obs}, Y_{mis}) denote a $n \times (p + 1)$ matrix of the observable growth histories of n lake trout from the same size category (i.e., their sizes at first capture were approximately equal). Except in the particular case of $p = 1$, the likelihood of our model's parameters given the observed data, $L(\mu, \Sigma | Y_{obs})$, cannot be factored into a product of independent, complete-data likelihoods, whose parameters are distinct subsets of μ and Σ (Schafer 1997, p. 16). Therefore, it is generally infeasible to compute estimates of model parameters by directly maximizing $L(\mu, \Sigma | Y_{obs})$. Indirect methods, such as the expectation-maximization algorithm or stochastic sampling (specifically, data augmentation), must be used to compute meaningful summaries of the observed-data likelihood (Schafer 1997, sections 5.3 and 5.4).

In our analysis of the sizes of recaptured lake trout, we used the expectation-maximization algorithm to compute maximum likelihood estimates of μ and Σ. These estimates provided starting values for data augmentation, which yields a sample of size m from the joint posterior distribution of model parameters and missing data, $p(\mu, \Sigma, Y_{mis} | Y_{obs})$ (see Appendix for computational details). An unbiased estimate of $E(Q|Y_{obs})$ may be computed using the values of μ in the simulated sample

$$\tilde{Q} = \frac{1}{m} \sum_{t=1}^{m} Q^{(t)}$$

where $Q^{(t)} = (\mu_1^{(t)} - \mu_0^{(t)}, \ldots, \mu_p^{(t)} - \mu_0^{(t)})'$ denotes the vector of mean growth increments in the tth draw of the simulated sample. However, the posterior draws of Y_{mis} also can be used to compute a Rao-Blackwellized estimate of $E(Q|Y_{obs})$ that has equal or smaller variance than $\tilde{Q}$ and is also unbiased (Schafer 1997, pp. 98–100). Therefore, we computed the Rao-Blackwellized estimate as follows:

$$(1.1) \quad \bar{Q} = \frac{1}{m} \sum_{t=1}^{m} E(Q | Y_{obs}, Y_{mis}^{(t)}) = \frac{1}{m} \sum_{t=1}^{m} \hat{Q}^{(t)}$$

where $\hat{Q}^{(t)} = (\bar{y}_1^{(t)} - \bar{y}_0, \ldots, \bar{y}_p^{(t)} - \bar{y}_0)'$ and $\bar{y}_j^{(t)}$ is the mean size of fishes recaptured in year j. Note that $\bar{y}_j^{(t)}$ is based on the observed sizes of fish that were recaptured in year j *and* on the imputed sizes of fish that were at large, but not seen, in year j.

To compute confidence intervals for Q, we require an estimate of the $p \times p$ covariance matrix of $\bar{Q}$. Using results of probability theory for calculating unconditional variances from conditional expectations, Rubin (1987, chapter 3) showed that an estimate of the covariance matrix of $\bar{Q}$ depends on the average of the conditional covariance matrices estimated for each of the m sets of complete data $(Y_{obs}, Y_{mis}^{(t)})$ and on the variation among the m complete-data posterior means, $\hat{Q}^{(t)}$. Specifically, an estimate of the total covariance matrix is

$$(1.2) \quad T = \bar{U} + (1 + m^{-1})B$$

where the average of the within-imputation conditional variance matrices is

$$(1.3) \quad \bar{U} = \frac{1}{m} \sum_{t=1}^{m} V(Q | Y_{obs}, Y_{mis}^{(t)}) = \frac{1}{mn} \sum_{t=1}^{m} (-1_p, I_p) S^{(t)} (-1_p, I_p)'$$

and the variation among complete-data posterior means is

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$$(1.4)\quad B = \frac{1}{(m-1)}\sum_{t=1}^{m}(\hat{Q}^{(t)} - \overline{Q})(\hat{Q}^{(t)} - \overline{Q})'.$$

In eq. 1.3, 1_p is a p-dimensional vector of ones, I_p is a $p \times p$ identity matrix, and $S^{(t)}$ is the sample covariance matrix of the observed and imputed sizes of fish in the tth draw of the simulated posterior distribution of Y_{mis}. We use eqs. 1.1–1.4 to calculate a $100(1-\alpha)\%$ confidence interval for the average growth increment of lake trout recaptured after j years at liberty as follows:

$$(1.5)\quad \overline{Q}_j \pm t_{\nu,1-\alpha/2}\sqrt{T_{jj}}$$

where the degrees of freedom of the t distribution is (Rubin 1987, chapter 3)

$$\nu = (m-1)\left[1 + \frac{\overline{U}_{jj}}{(1+m^{-1})B_{jj}}\right]^2$$

illustrating the dependence of ν on the size of the simulated sample and on the relative increase in variance due to the incompletely observed growth histories.

Comparison of growth increment estimates

We wish to compare the estimated growth increments of lake trout that were marked and recaptured during the prerefuge period with those of lake trout that were marked and recaptured during the postrefuge period. Provided the comparison includes lake trout that were the same size initially (i.e., in the same size category at first capture), we may use a likelihood ratio test, modified for incomplete data (Meng and Rubin 1992), to assess whether the mean growth histories of these two groups of fish are significantly different. Let (Y_{obs}, Y_{mis}) be the $(n_1 + n_2) \times (p + 1)$ matrix of observable sizes (the first n_1 rows correspond to fish from group 1 (prerefuge); the remaining rows correspond to fish from group 2 (postrefuge)). We compare a model in which mean sizes in each year of recapture differ between the two groups ($Y_{i\in\{1,\ldots,n_1\}} \sim N(\mu_1, \Sigma)$; $Y_{i\in\{n_1+1,\ldots,n_1+n_2\}} \sim N(\mu_2, \Sigma)$) with a model where the mean sizes at recapture are identical for both groups ($Y_{i\in\{1,\ldots,n_1+n_2\}} \sim N(\mu, \Sigma)$). The complete-data likelihood ratio statistic for testing H_0: $\mu_1 = \mu_2 = \mu$ is

$$(1.6)\quad d_L = 2[l(\hat{\mu}_1, \hat{\mu}_2, \hat{\Sigma}_*|Y_{obs}, Y_{mis}) - l(\hat{\mu}, \hat{\Sigma}|Y_{obs}, Y_{mis})]$$

where $\hat{\mu}_1$, $\hat{\mu}_2$, and $\hat{\Sigma}_*$ are parameter estimates that maximize the complete-data log likelihood $l(\mu_1, \mu_2, \Sigma|Y_{obs}, Y_{mis})$ and $\hat{\mu}$ and $\hat{\Sigma}$ are estimates that maximize the complete-data log likelihood $l(\mu, \Sigma|Y_{obs}, Y_{mis})$.

To simplify notation, let $\hat{\psi}_* = \{\hat{\mu}_1, \hat{\mu}_2, \hat{\Sigma}_*\}$ and $\hat{\psi} = \{\hat{\mu}, \hat{\Sigma}\}$. Therefore, using this notation, the likelihood ratio statistic in eq. 1.6 is

$$d_L(\hat{\psi}_*, \hat{\psi}|Y_{obs}, Y_{mis}) = 2[l(\hat{\psi}_*|Y_{obs}, Y_{mis}) - l(\hat{\psi}|Y_{obs}, Y_{mis})]$$

The test statistic proposed by Meng and Rubin (1992) requires computation of the maximum likelihood estimates, $\hat{\psi}_*$ and $\hat{\psi}$, and the likelihood ratio test statistic for each of the m sets of complete data created by data augmentation. Denoting these by $\hat{\psi}_*^{(t)}$, $\hat{\psi}^{(t)}$, and $d_L(\hat{\psi}_*^{(t)}, \hat{\psi}^{(t)}|Y_{obs}, Y_{mis}^{(t)})$, respectively, for the tth complete data set, we compute their average values over all m data sets as follows:

$$\overline{\psi}_* = \frac{1}{m}\sum_{t=1}^{m}\hat{\psi}_*^{(t)}$$

$$\overline{\psi} = \frac{1}{m}\sum_{t=1}^{m}\hat{\psi}^{(t)}$$

$$\overline{d}_L = \frac{1}{m}\sum_{t=1}^{m} d_L(\hat{\psi}_*^{(t)}, \hat{\psi}^{(t)}|Y_{obs}, Y_{mis}^{(t)}).$$

We also compute an average of the complete-data likelihood ratio statistic evaluated at $\overline{\psi}_*$ and $\overline{\psi}$ as follows:

$$\tilde{d}_L = \frac{1}{m}\sum_{t=1}^{m} d_L(\overline{\psi}_*, \overline{\psi}|Y_{obs}, Y_{mis}^{(t)}).$$

The test statistic proposed by Meng and Rubin (1992) for testing H_0: $\mu_1 = \mu_2 = \mu$ is

$$D_L = \frac{\tilde{d}_L}{(p+1)(1+r_L)}$$

where

$$r_L = \frac{m+1}{(p+1)(m-1)}(\overline{d}_L - \tilde{d}_L).$$

The p value associated with D_L is

$$p = P(F_{p+1,\nu_L} \geq D_L)$$

where the degrees of freedom parameter of the reference distribution is

$$\nu_L = \begin{cases} 4 + (q-4)[1 + (1-2q^{-1})r_L^{-1}]^2 \text{ if } q=(p+1)(m-1) > 4 \\ (q/2)[1 + (p+1)^{-1}](1 + r_L^{-1})^2 \quad \text{otherwise} \end{cases}$$

Results

To accommodate size-specific differences in growth of lake trout, we estimated the mean growth increments of individuals in six size categories: ≤60, 60–65, 65–70, 70–75, 75–80, and >80 cm. Fish were assigned to a size category based on their length at first capture, and most were between 60 and 80 cm at first capture (Fig. 2). Although the size distributions of hatchery and wild lake trout were similar during the pre- and post-refuge periods, hatchery fish tended to be somewhat larger during the prerefuge period (Fig. 2).

To determine the number of years available for estimating mean growth increments, we examined annual recapture rates of lake trout in each of their first 6 years at liberty (Table 2). Within each recapture period (pre- and post-refuge), wild and hatchery fish exhibited similar patterns in the distribution of recaptures with time at liberty. Although more fish were recaptured over more years during the postrefuge period, this may simply be a result of the difference in number of years available for actual observation (the maximum for the prerefuge period was 6, and the maximum for the postrefuge period was 21). In this study, recapture rates of lake trout on the spawning grounds were influenced by apparent survival and by factors affecting catchability such as behavior (particularly spawning behavior). For a given recapture period, hatchery and wild fish appeared to be equally susceptible to capture; thus, growth increment estimates are not thought to have been influenced by differences in behav-

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Fig. 2. Size distributions of lake trout at first capture. (*a*) Wild fish, prerefuge period; (*b*) hatchery fish, prerefuge period; (*c*) wild fish, postrefuge period; (*d*) hatchery fish, postrefuge period.

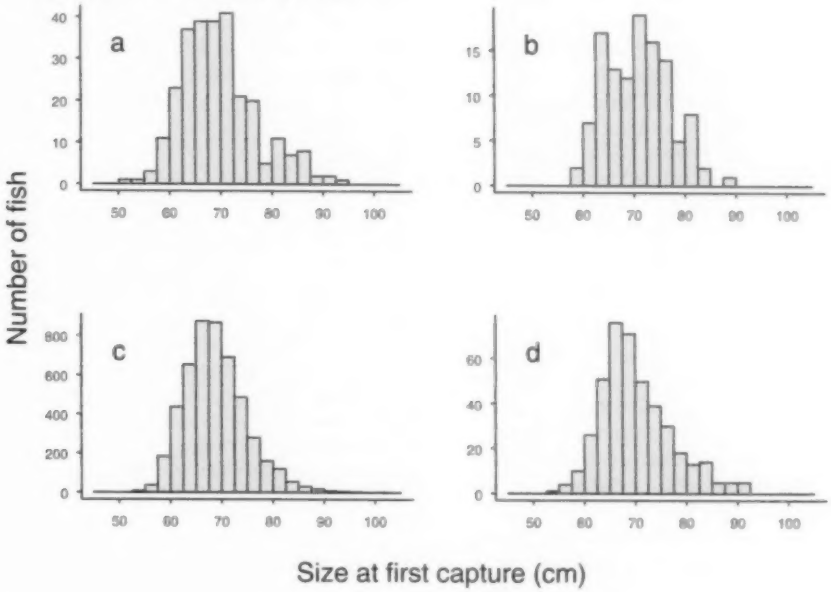

Table 2. Annual recapture rates (% of lake trout that were recaptured at least once) in each of the first 6 years at liberty.

			No. of years at liberty					
Fish origin	Recapture period	No. of fish	1	2	3	4	5	6
Hatchery	Prerefuge	116	70.7	24.1	12.1	2.6	0.0	0.0
Wild	Prerefuge	272	72.4	23.5	7.7	1.8	3.3	0.4
Hatchery	Postrefuge	418	38.8	29.9	17.2	13.2	8.4	3.8
Wild	Postrefuge	4923	39.6	31.4	19.2	11.7	8.0	5.2

iors or apparent survival. We included for statistical analysis only the sizes of lake trout observed in their first 3 years at liberty owing to the limited number of recaptures in years 4–6 of the prerefuge period.

Wild fish

Mean growth increments of wild lake trout in each of their first 3 years at liberty were significantly different during the pre- and post-refuge periods in five of the six size categories examined (Table 3). Estimates of growth during the prerefuge period generally exceeded estimates of growth during the postrefuge period (Fig. 3). The smaller numbers of fish tagged and recaptured during the prerefuge period contribute to wider confidence intervals. Note also that confidence intervals are narrowest for mean growth increments estimated after 1 year at liberty and widest for those after 3 years at liberty, reflecting an increase in uncertainty as recapture rates declined (and missingness increased) with time at liberty (Table 2).

Mean growth increments of wild fish in a given size category decreased with increasing time at liberty (Fig. 3). This result is consistent with the growth pattern specified by the VB model, which is based on size at age. Although we did not explicitly account for fish age, our approach accommodated the pattern of decreasing growth with increasing age.

Table 3. Values of p for testing if mean growth increments of lake trout during their first 3 years at liberty were equal during the pre- and post-refuge periods.

Size at first	Wild			Hatchery		
capture (cm)	n_{pre}	n_{post}	p	n_{pre}	n_{post}	P
<60	16	228	0.02	2	15	na
60–65	60	1089	<0.01	24	77	0.26
65–70	78	1744	<0.01	25	147	<0.01
70–75	62	1178	<0.01	35	89	<0.01
75–80	25	440	0.37	19	48	0.56
>80	31	244	<0.01	11	42	0.10

Note: Numbers of fish from each size category in the pre- and post-refuge periods are indicated by n_{pre} and n_{post}, respectively. na, number of fish insufficient to compute a p value.

In addition, the variance of the mean growth increment increased with time (Fig. 3), but this likely reflected fewer recaptures after 2 or more years at liberty. We also observed a reduction in estimated annual growth with increasing size at first capture. Thus, larger (older) fish grew at slower rates than the smaller (younger) fish in our samples. Together, these results suggest that growth of wild lake trout may be

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Fig. 3. Size-specific estimates and 95% confidence intervals of individual growth increments of wild lake trout during the (*a*) prerefuge and (*b*) postrefuge periods. Estimates of growth after 1, 2, or 3 years at liberty are distinguished by symbol type (circle, square, or triangle, respectively).

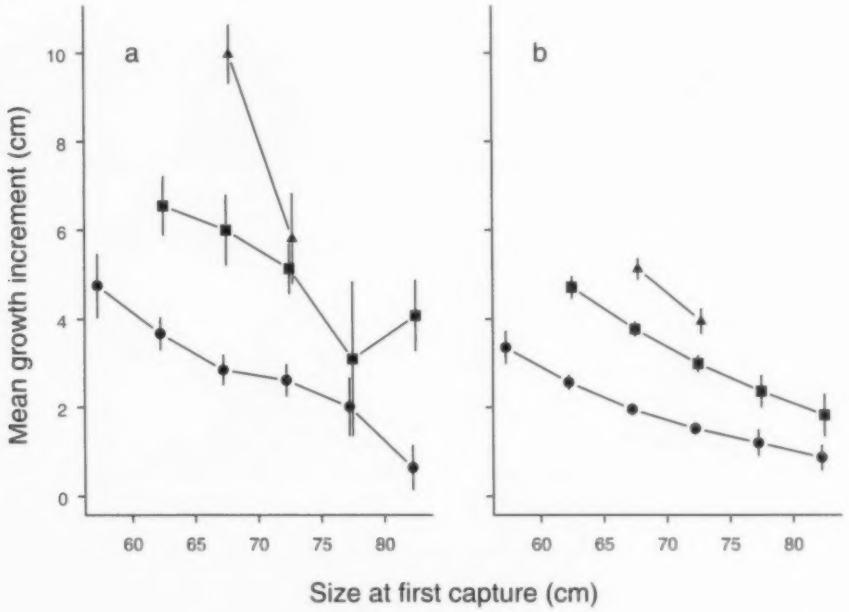
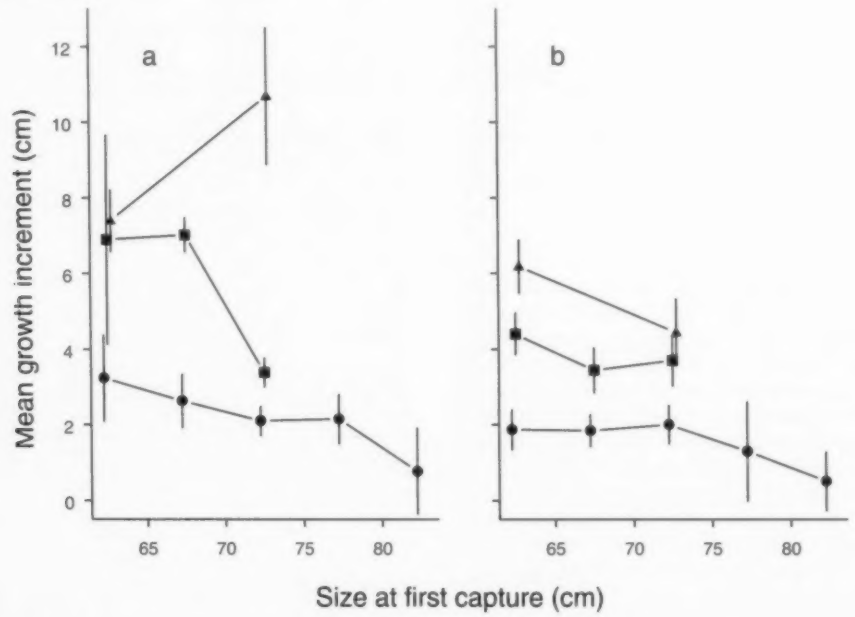


Fig. 4. Size-specific estimates and 95% confidence intervals of individual growth increments of hatchery-reared lake trout during the (*a*) prerefuge and (*b*) postrefuge periods. Estimates of growth after 1, 2, or 3 years at liberty are distinguished by symbol type (circle, square, or triangle, respectively).



adequately described by the VB growth model if it becomes possible to obtain accurate ages.

Hatchery fish

Mean growth increments of hatchery-raised lake trout in each of their first 3 years at liberty were significantly different during the pre- and post-refuge periods in only two of the six size categories (Table 3). In both of these cases, growth increments were greater in the prerefuge period than in the postrefuge period (Fig. 4). Due to the increased vari-

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ability associated with smaller numbers of fish and fewer recaptures, growth patterns of hatchery fish were more difficult to discern but appeared to be similar to those of wild fish. For example, during the postrefuge period, mean growth increments of hatchery fish in the most abundant size categories (65–70 and 70–75 cm) decreased with increasing time at liberty (Fig. 4).

Discussion

Size-at-age-based growth estimates

In the Laurentian Great Lakes, growth of lake trout (or simply mean length at age) is often reported as an indicator of the general condition of the population, and only gross comparisons of growth have been attempted. Mean length at age appears to differ among populations in different areas of a lake (e.g., see Hansen 1994) and fluctuations in average length appear to occur through time; however, no formal statistical analyses have been attempted in these investigations. Yet, lake trout growth is an important consideration for fishery managers because one of the fish community objectives for Lake Superior is to "maintain a predator–prey balance in the lake that allows the normal growth of lake trout" (Busiahn 1990). Furthermore, in the restoration plan for Lake Superior lake trout, Hansen (1996) recommended that a "more meaningful expression of lake trout growth than length at age 7" be developed because average length at age was thought to be insensitive to true changes in growth.

Individual growth of lake trout may be affected by a number of factors including population abundance, composition of the forage base, forage abundance, and water temperature. In Minnesota waters of Lake Superior, growth rates of lake trout were shown to be sensitive to prey availability (Negus 1995). This sensitivity is relevant to the management of lake trout, which now may compete for food resources with nonindigenous salmonids in Lake Superior. The bioenergetics approach is being extended to encompass lake trout populations throughout Lake Superior (Negus 1995, but such modeling clearly requires a better understanding of lake trout growth and corroboration by field studies of growth (Ney 1993). For example, Negus (1995) noted that the observed discrepancy between bioenergetic estimates of biomass consumed by predators and estimates of available prey derived from bottom-trawl surveys may be partially explained by changes in lake trout growth rates, which were not modeled. Representative data (i.e., model inputs) are critical to improving the accuracy of model outputs (Ney 1993). Our study provides additional insight into growth dynamics of the Gull Island Shoal population as well as further evidence for growth differences among hatchery and wild lake trout.

Mark–recapture-based growth estimates

Growth analyses are often based on measures of size at age, but scale-based estimates of age (and hence, growth rates) are biased for large, mature lake trout (Schram and Fabrizio 1998). Age determinations of mature lake trout are more reliable when otoliths are used to determine age. However, this requires sacrificing the fish—an untenable proposition when rehabilitation is sought. In contrast, estimates of growth derived from mark–recapture studies are not affected by uncertainties in age determinations. Unfortunately, in the absence of information on ages of marked fish, mark–recapture-based growth estimates are not interchangeable with growth estimates derived from traditional length at age analyses (Francis 1988).

One of the assumptions necessary to estimate growth of individual fish in a mark–recapture study is that the tagging procedure or tag have no effect on growth. The effects of anchor tags on the growth of mature lake trout have not been studied, but we believe that such effects were minor and possibly undetectable in our study. Growth rates of brook trout (*Salvelinus fontinalis*), a closely related species, were suppressed only during the first 3 months after tagging with anchor tags (Floy tag FD-67); thereafter, growth rates of tagged fish were similar to those of untagged fish (Carline and Brynildson 1972). Studies that reported retardative effects of tags shared one or more of the following conditions: (*i*) tag type or size was inappropriately matched to fish size (e.g., Manire and Gruber 1991), (*ii*) tags were applied to young life stages, which have relatively high growth rates (e.g., 2-year-old lake trout; Eschmeyer 1959), (*iii*) tags (jaw tags) interfered with feeding (e.g., Warner 1971), or (*iv*) stress due to spawning (Gunn et al. 1979) or the presence of wounds (e.g., Carline and Brynildson 1972) contributed to decreased growth rates. In addition, retardative effects are likely to be significant only when growth is studied over short (<1 year) periods of time (Carline and Brynildson 1972). In the absence of these conditions and when tagging effects are assessed over long periods of time, growth of tagged and untagged individuals is similar.

An additional assumption in our comparison of growth histories of hatchery and wild fish is that fin clipping does not affect growth. In our study, only the hatchery-reared lake trout were subjected to this treatment, which occurred at the yearling stage just before the fish were stocked. Fin clipping, a common method of marking large numbers of fish, generally has no significant effect on fish growth but may reduce growth rates in young fish (<90 mm; Coble 1967). Extensive testing with fingerling lake trout <90 mm in length (average size ranged from 74 to 83 mm) indicated no appreciable effect on growth when the following fins were clipped: adipose, dorsal, right pectoral, or right pelvic (Shetter 1950). In these studies, lake trout growth rates were monitored in hatchery conditions over periods ranging from 2 to 4.5 years (Shetter 1950). Additionally, in the only study of its kind, the combination of fin clipping and anchor tagging appears to have no significant effect on growth of largemouth bass (*Micropterus salmoides*) (Tranquilli and Childers 1982). Together, these studies support the notion that fin clipping and anchor tagging of adult lake trout in Lake Superior have no effect on growth as estimated from our tagging experiment.

Our observations on decreasing growth increment with increasing time at liberty and with increasing size at capture suggest that growth rates of mature lake trout decline with age and size. Mature lake trout at Gull Island Shoal (particularly wild fish) continued to increase in length (in some cases appreciably) even after 2, 3, or more years at liberty. Even during the postrefuge period, when growth was relatively slower, wild fish in our largest size category grew by 0.87 cm (±0.14 SE) during the first year at liberty. This suggests that mature lake trout may still experience appreciable

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growth and that growth rates of lake trout in the Gull Island Shoal area permit these fish to attain large sizes.

Are our conclusions about growth of lake trout robust to the discretization of size at capture and years at liberty? If the discretization of sizes into categories is too coarse, many ages of fish may be included in each category, and we will be unable to detect changes in growth over time. Conversely, if size categories are too narrow and contain too few fish, our growth increment estimates will be imprecise, again leading to difficulties in detecting changes in growth. We believe that the use of 5-cm size categories for mature lake trout appears to strike a balance between the risks of including too many age-classes and excluding too many fish. In our study, even the largest mature lake trout exhibited annual growth increments that were significantly greater than zero (as noted earlier).

Dynamics of individual growth of Lake Superior lake trout

In Lake Superior, the diet of lake trout shifted from one dominated by lake herring (*Coregonus artedi*) and deepwater ciscoes (*Coregonus* spp.) before the 1950s to one dominated by rainbow smelt (*Osmerus mordax*) by the early 1960s (Conner et al. 1993). This switch paralleled observed changes in the composition of prey fishes in the lake. Although lake herring are now more abundant than rainbow smelt, lake trout preferentially consume rainbow smelt (Mason et al. 1998). For example, between 1985 and 1987 in the Apostle Islands area, rainbow smelt composed about 27% of the prey fish biomass, but lake trout diets were 66–78% rainbow smelt by weight (Conner et al. 1993). Growth (as indicated by mean length at age) of lake trout from Michigan waters of Lake Superior appeared to decline between 1980 and 1989, somewhat coincident with the decline in rainbow smelt abundance that began in the late 1970s (Hansen 1994). However, a closer examination of the relationship between prey fish abundance and lake trout growth in Lake Superior revealed that growth rates of hatchery fish during 1979–1984 were poorly associated with abundance of prey fish (Eby et al. 1995). Furthermore, consumption rates of lake trout from several Great Lakes were shown to have been similar even though prey fish abundance differed by a factor of 100 among lakes (Eby et al. 1995). An age-structured model that combined bioenergetics modeling and foraging theory also supported the notion that growth of lake trout, particularly for fish 4 years and older, was not entirely regulated in a density-dependent manner (Mason et al. 1998).

Effect of the Gull Island Shoal refuge

Our observations of the growth dynamics of lake trout at Gull Island Shoal cannot be used to determine whether density-dependent or density-independent mechanisms were responsible for the changes in individual growth rates; however, the evidence does indicate that growth responses of hatchery and wild fish were similar. After the refuge was established, the lake trout population at Gull Island Shoal increased in abundance and average annual growth increments of individual fish decreased significantly. This reduction in growth may have been associated with a reduction in food availability, but we cannot be certain about the cause(s) of reduced food for lake trout given the limitations of our observational study and the paucity of other supporting evidence. More specifically, we are unsure whether food availability may have decreased because of the increased abundance of lake trout during the postrefuge period (a density-dependent response) or because of changes in environmental conditions that just happened to coincide with the establishment of the refuge (a density-independent response).

Refuges, sanctuaries, reserves, and other "no-take" areas are increasingly considered as options for managing overexploited fisheries (e.g., Johnson et al. 1999) or as an aid to rehabilitating native species (e.g., Schram et al. 1995). An expected proximate result is a decrease in fishing mortality rates allowing slow-growing fish to achieve maturity and spawn. The ultimate results of such sanctuaries—increased population abundance, expanded size and age structures, dispersal of recruits to areas outside the sanctuary—have been assessed only recently. In addition to our study of lake trout, Johnson et al. (1999) reported that in coastal marine areas protected from fishing, several species of large, slow-growing fishes increased in abundance and average size. Growth rates were not estimated, so it is unknown if the observed changes in the population were accompanied by changes in individual growth. In Gull Island Shoal, Lake Superior, the establishment of a refuge was followed by an increase in abundance of lake trout and a decrease in their individual rates of growth. Further studies of the potential ultimate effects of refuges and sanctuaries may provide additional insights into the dynamics of recovering fish populations.

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Appendix. Simulating a sample from the joint posterior distribution of model parameters and unobserved sizes of fish

We use a stochastic sampling algorithm called data augmentation (Tanner and Wong 1987; Gelfand and Smith 1990) to simulate an arbitrarily large number of draws from the joint posterior distribution of model parameters and missing data (i.e., unobserved sizes of fish). Although the joint posterior density $p(\mu, \Sigma, Y_{mis}|Y_{obs})$ is difficult to compute or sample directly, it may be sampled indirectly by computing recursive draws from conditional distributions that are easier to sample. For example, given an initial guess of the parameter values, say $\mu^{(t)}$ and $\Sigma^{(t)}$, data augmentation begins by drawing a random value from the conditional predictive distribution of Y_{mis}:

$$\text{(A1)} \quad Y_{mis}^{(t+1)} \sim p(Y_{mis}|Y_{obs}, \mu^{(t)}, \Sigma^{(t)}).$$

Then, conditioning on $Y_{mis}^{(t+1)}$, new values of μ and Σ are randomly drawn from the complete-data posterior:

$$\text{(A2)} \quad \mu^{(t+1)}, \Sigma^{(t+1)} \sim p(\mu, \Sigma|Y_{obs}, Y_{mis}^{(t+1)}).$$

Tanner and Wong (1987) showed that repeating eqs. A1 and A2 from arbitrary starting values $\mu^{(0)}$ and $\Sigma^{(0)}$ yields a stochastic sequence

$$\{(\mu^{(t)}, \Sigma^{(t)}, Y_{mis}^{(t)}): t = 1, 2, \ldots\}$$

whose stationary distribution is the joint posterior $p(\mu, \Sigma, Y_{mis}|Y_{obs})$.

The data augmentation algorithm is particularly easy to implement for our complete-data model of the observable sizes of lake trout (i.e, the multivariate normal model). In the

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absence of prior information about the values of μ or Σ, we adopt a uniform prior for μ and Jeffrey's prior for Σ (i.e., $p(\mu, \Sigma) \propto \det(\Sigma)^{-(p+2)/2}$), as recommended by Schafer (1997, p. 154). As a consequence of these priors, a draw from the complete-data posterior (eq. A2) is obtained by computing random draws from the inverse-Wishart and normal distributions (in that order) as follows:

$$\Sigma^{(t+1)} | Y_{obs}, Y_{mis}^{(t+1)} \sim \text{Inverse-Wishart}\,(n-1, (nS)^{-1})$$

$$\mu^{(t+1)} | \Sigma^{(t+1)}, Y_{obs}, Y_{mis}^{(t+1)} \sim N(\bar{y}, n^{-1}\Sigma^{(t+1)})$$

wherein $\bar{y}$ and S denote the sample mean vector and covariance matrix, respectively, of the matrix of observed and imputed sizes of fish from the $(t + 1)$th random draw.

A random draw from the conditional predictive distribution (eq. A1) is also relatively easy to compute because the rows of (Y_{obs}, Y_{mis}), which correspond to growth histories of individual fishes, are conditionally independent; therefore, a random draw from eq. A1 is obtained by drawing

$$\text{(A3)} \quad y_{i(mis)}^{(t+1)} \sim p(y_{i(mis)} | y_{i(obs)}, \mu^{(t)}, \Sigma^{(t)})$$

independently for $i = 1, \ldots, n$. For our model of lake trout growth histories, Schafer (1997, p. 181) showed that eq. A3 represents a draw from a conditionally multivariate normal distribution whose mean and covariance matrix may be computed in terms of $y_{i(obs)}$, $\mu^{(t)}$, and $\Sigma^{(t)}$. Concise expressions for the conditional mean and covariance matrix are difficult to write down in any generality because they depend on the particular elements of y_i that are observed; however, the parameters of the conditional multivariate normal are easily specified and evaluated in terms of sweep operators (Schafer 1997, pp. 159–163, 181).

We computed a sample of $m = 100$ draws from the joint posterior density $p(\mu, \Sigma, Y_{mis} | Y_{obs})$ using a software package called NORM, which implements the data augmentation algorithm described above and is available at the World Wide Web address www.stat.psu.edu/~jls/ (Schafer 1997). The data augmentation algorithm was initialized with maximum likelihood estimates of μ and Σ, which were computed using expectation-maximization in NORM. To help ensure that data augmentation had been run sufficiently long to have converged to a stationary distribution, we ran the algorithm for 2000 draws and discarded the first 1000 draws as a "burn-in" period. Our simulated sample was obtained by selecting every tenth draw of the remaining 1000 draws to avoid dependencies between successive elements of the sequence of model parameters and imputed values of Y_{mis}.

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Grazing dynamics of *Euphausia spinifera* in the region of the Subtropical Convergence and the Agulhas Front

Renzo Perissinotto, Patrick Mayzaud, Jean-Philippe Labat, and Suzanne Razouls

Abstract: The feeding ecophysiology of the subtropical euphausiid *Euphausia spinifera* was investigated in the Indian sector of the Southern Ocean during January–February 1999. Gut pigment levels varied from 1.7 to 40.6 ng chlorophyll *a* (Chl *a*) equiv.·individual^{-1} in adults and from 0.3 to 9.3 ng Chl *a* equiv.·individual^{-1} in juveniles. Highest levels were observed at the Subtropical Convergence (Chl *a* concentrations 0.4–0.6 μg·L^{-1}) and minima in the area north of the Agulhas Front (Chl *a* concentrations 0.2–0.3 μg·L^{-1}). Gut evacuation rates ranged between 0.59 and 0.96·h^{-1}. Gut pigment destruction levels were among the highest ever recorded in euphausiids, with 94.2–98.5% of total pigments ingested converted to nonfluorescing end products. Size-selectivity experiments with natural phytoplankton showed that *E. spinifera* ingests mainly cells in the 0.7- to 20-μm range. The grazing dynamics of this important species are compared with those of subantarctic (*Euphausia vallentini*) and Antarctic krill (*Euphausia superba*). Individual ingestion rates, estimated from the autotrophic component of its diet (2.78–4.46 μg Chl *a* equiv.·individual^{-1}·day^{-1}), were just sufficient to account for respiratory requirements. While *E. spinifera* is clearly able to ingest large amounts of heterotrophic prey, it is not known to what extent these contribute to its total energy budget.

Résumé : Nous avons étudié l'écophysiologie de l'alimentation chez l'euphausiidé subtropical *Euphausia spinifera* dans le secteur indien de l'Océan Austral en janvier et février 1999. Les quantités de pigments dans le tube digestif variaient de 1,7 à 40,6 ng equiv.-chlorophylle *a* (Chl *a*)·individu^{-1} chez les adultes et de 0,3 à 9,3 ng equiv.-Chl *a*·individu^{-1} chez les jeunes. Les valeurs les plus élevées ont été observées à la Convergence subtropicale (concentrations de Chl *a* : 0,4–0,6 μg·L^{-1}) et les plus basses dans la région au nord du Front des Agulhas (concentrations de Chl *a* : 0,2–0,3 μg·L^{-1}). Les taux d'évacuation du tube digestif variaient de 0,59 à 0,96·h^{-1}. Les taux de destruction des pigments dans le tube digestif étaient parmi les plus élevés jamais enregistrés chez les euphausiidés: 94,2–98,5% des pigments totaux ingérés se transformaient en produits terminaux non-fluorescents. Des études de sélectivité de la taille du phytoplancton naturel montrent qu'*E. spinifera* ingère surtout des cellules dans l'intervalle de tailles 0,7–20 μm. La dynamique de broutage de cette espèce importante a pu être comparée à celles du krill subantarctique (*Euphausia vallentini*) et du krill antarctique (*Euphausia superba*). Les taux d'ingestion d'un individu, estimés d'après la fraction autotrophe de son régime (2,78–4,46 μg equiv.-Chl *a*·individu^{-1}·jour^{-1}), suffisent tout juste à combler ses besoins respiratoires. Bien qu'*E. spinifera* soit capable d'ingérer de grandes quantités de proies hétérotrophes, on ne sait pas dans quelle mesure celles-ci contribuent à son bilan énergétique total.

[Traduit par la Rédaction]

Introduction

Euphausia spinifera is a subtropical euphausiid species found in all three major ocean basins (Boden 1954; Mauchline and Fisher 1969; Kirkwood 1982). It occurs in the Southern Hemisphere at latitudes from about 42 to 30°S and in a temperature range of 6–19°C (Fig. 1) (Baker 1965; Lomakina 1966; Kirkwood 1982). The size range of juveniles and adults of the species is 10–20 and 21–29 mm, respectively (Boden 1954). Stomach content analysis suggests that the species is able to ingest heterotrophic material, such as crustaceans and foraminiferans (Ponomareva 1976; Mauchline 1980). Nothing has been published on the potential contribution of autotrophic material to the overall energy budget of the species. The lack of any reported large phytoplankton material by Ponomareva (1976) suggests either that the species is strictly carnivorous or that it feeds on small-sized phytoplankton cells, not easily recognizable with the microscopic technique used by Ponomareva (1976). The species has been recorded in large densities in the stomach content of sei (Baker 1965), humpback (Dall and Dunstan 1957), and fin whales (Bannister and Baker 1967), suggesting that at times, it forms swarms. The role and importance of *E. spinifera* in the food web of the subtropical marine ecosystem are mostly unknown.

During the ANTARES-4 cruise of the French research

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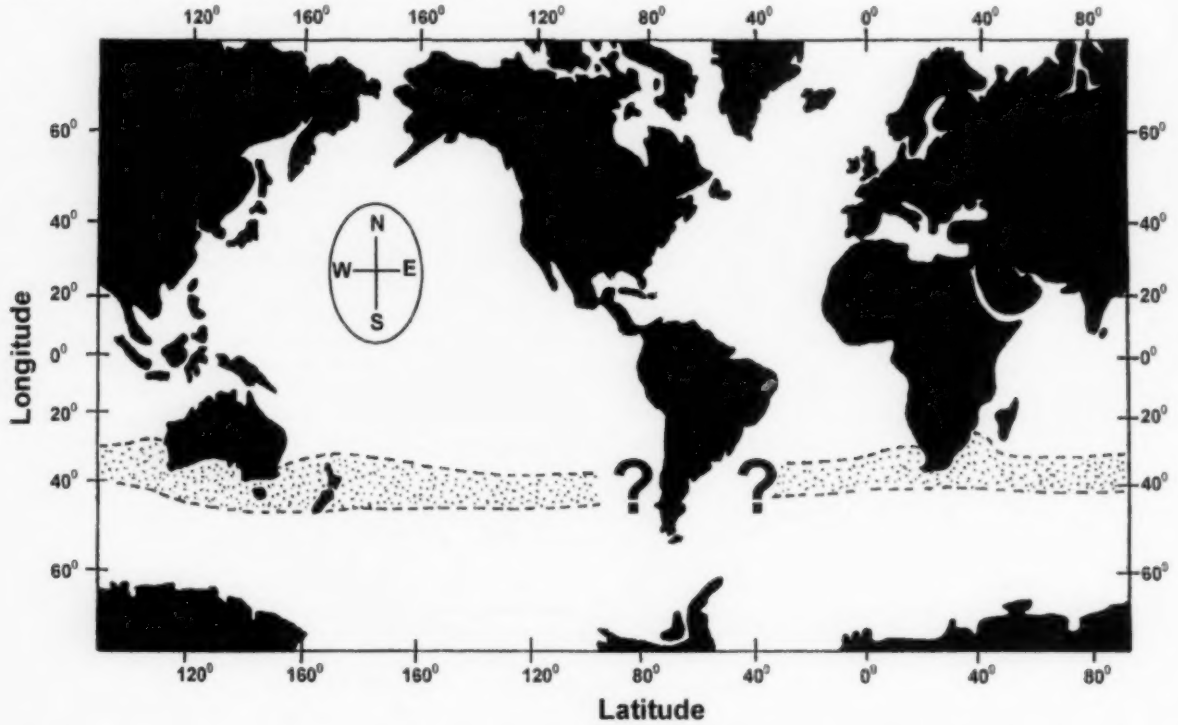
Fig. 1. Disribution range of *E. spinifera* in the world ocean (adapted from Mauchline and Fisher (1969) and Kirkwood (1982)). Question marks denote areas for which no information is available.



ship *Marion-Dufresne* in January–February 1999, several transects and stations were occupied in the region to the north of the Subtropical Convergence in the Indian sector of the Southern Ocean. On several occasions, dense catches of *E. spinifera* were made between the convergence zone and the Agulhas Front. Initial observations made onboard suggested a potential large contribution of algal material to the gut contents of this species. This prompted the investigation of the feeding ecology of *E. spinifera* in relation to resource availability in the region and its energy expenditure. Here, we report results on in situ grazing rates for this species on the autotrophic food component available in the region at the time of the survey. These are then compared with its basic metabolic (respiratory) requirements in order to assess the potential role of heterotrophic prey in its total energy budget.

Materials and methods

The ANTARES-4 survey was carried out during the period 5 January – 23 February 1999 in the Indian sector of the Southern Ocean (Fig. 2). The survey consisted mainly of an intensive grid of lines and three long stations (stations 3, 7, and 8), each occupied for a period of 3–6 days. *Euphausia spinifera* was collected in large densities only in the region north of the Subtropical Convergence, mainly at stations P3, G4, 7, and 8 (Fig. 2).

Euphausia spinifera and zooplankton samples were collected using three different types of gear: a 160-cm-diameter Omori net (1000-μm mesh), a 67-cm-diameter Bongo net × 2 (200- and 500-μm mesh), and a 57-cm-diameter WP-2 × 3 (200-μm mesh) system. Tows with the Omori and Bongo nets were made obliquely from 100–200 m to the surface, while the WP-2 system was towed vertically from 200 m to the surface. At most stations, only one net tow was made in order to investigate the spatial variability in biomass and community structure in the survey area. At longer stations, net tows were repeated at 4- to 5-h intervals to study diel variations in zooplankton abundance and grazing rates. Biomass estimates of representative *E. spinifera* individuals were obtained as dry weights by oven-drying specimens at 65°C for 36 h.

Samples for chlorophyll *a* (Chl *a*) determination were collected using a 24-bottle rosette (General Oceanics). Size-fractionated Chl *a* concentrations were obtained with parallel filtration of 500-mL samples through 10- and 2-μm polycarbonate filters (Nuclepore) and through GF/F glass fibre filters (Whatman) (M. Fiala, Observatoire Océanologique, B.P. 44, 66651 Banyuls-sur-mer Cedex, France, unpublished data). Occasionally, samples were first passed through a 20-μm nylon filter in order to isolate the largest components. Pigments were extracted from each filter in 90% acetone and fluorescence measured on a Perkin-Elmer MPF 66 spectrofluorometer (Fiala et al. 1998).

Feeding rates of *E. spinifera* on the autotrophic material available in the region were estimated using the latest version of the gut pigment technique. This consists of a combination of simultaneous measurements of gut pigment contents, gut evacuation rates, and gut pigment destruction (Perissinotto et al. 1997; Tirelli and Mayzaud 1998). Gut pigment contents were measured in freshly collected animals after 24 h of extraction in 8 mL of 90% acetone solution. The pigment content of the supernatant was measured after centrifugation with a Turner Designs fluorometer before and after acidification with 1 N HCl. Chl *a* and phaeopigment concentrations were added together and expressed in units of Chl *a* equivalents (Conover et al. 1986). Four to eight replicate individuals (generally one individual per tube) were used for pigment extraction and measurement from each tow.

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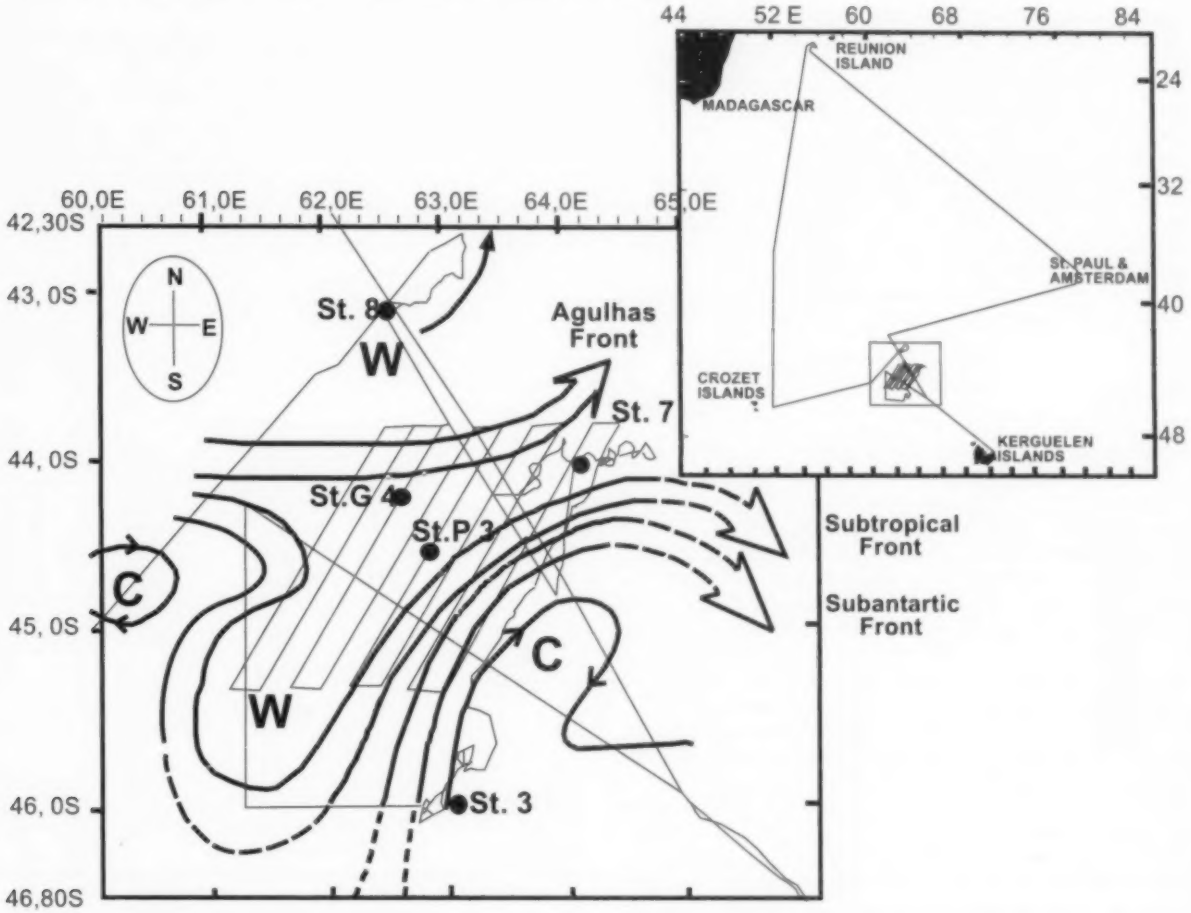
Fig. 2. Frontal systems and station positions during the ANTARES-4 cruise to the subantarctic and subtropical sectors of the Indian Ocean (courtesy of Y.-H. Park, Laboratoire d'Océanographie physique, Muséum National d'Histoire Naturelle, 43 rue Cuvier, 75005 Paris, France). Broken lines are used to indicate the probable position of the fronts in areas where no actual data were collected. The inset shows the entire cruise track covered between the 5 January and the 23 February 1999.



Gut evacuation rates were estimated by incubating animals freshly caught with night tows in 0.2-µm-filtered seawater. Small amounts of nonfluorescing cornstarch were added to keep the animals under continuous feeding conditions. This is a necessary step with euphausiids in order to displace previously ingested food (Willason and Cox 1987; Perissinotto and Pakhomov 1996). The incubation period was 2 h and the decline in gut pigment content was monitored at intervals of 10–15 min for the first hour and of 30 min thereafter. Gut evacuation rates (per hour) were derived from the slope of the regression of the natural log of gut pigments versus time (Dam and Peterson 1988). Loss of pigments to nonfluorescent end products by stomach digestion was estimated at the two long stations, 7 and 8. Four independent measurements of pigment loss were made on each occasion following the two-compartment protocol described by Perissinotto and Pakhomov (1996). Briefly, the method involves a comparison of the pigment budgets of a control versus an experimental parcel of natural seawater in which krill with empty stomachs are allowed to feed for 1 h. Any significant loss in the pigment budget from the experimental treatment is then attributed to gut destruction of phytoplankton pigments.

In the size-selectivity experiments, four replicates of two animals each were incubated in 1 L of natural seawater for 1 h in darkness and at ambient temperature. Duplicate water samples of 100 mL were taken from each bottle at the beginning and end of the incubation for Chl *a* and phaeopigment analysis. Four size-classes of phytoplankton (>20, 10–20, 2–10, and <2 µm) were obtained by multiple filtration on a Millipore manifold. Nitex (20 µm), Nuclepore (10 and 2 µm), and Whatman GF/F filters were used for this purpose. Pigments from the different filters were extracted for 24 h in 8 mL of 90% acetone and concentrations were calculated from fluorescence measured with a Turner Designs fluorometer.

Stomach content analysis was performed on 30 individuals, 10 each from stations G4, 7, and 8. Individuals included subadult/adult animals of 18–22 mm total length collected during day (n = 10, 11:30 local time) and night (n = 20, 23:35–00:50 local time). Stomachs were removed and dissected under a stereoscopic microscope; their contents were then placed on a hollow slide and the food items identified, sized, and counted using an Olympus inverted microscope at 400× magnification.

Respiration rates were measured using a method adapted from Teal and Halcrow (1962). Incubations were carried out at an in situ temperature of 13–14°C using animal densities of one individual per 55–75 mL over a time period of 6 h and in darkness. The vol-

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ume of the incubation vessel ranged from 55 mL (one animal) to 150 mL (two animals). Experiments were conducted in a temperature-controlled room with an accuracy of ±1°C. O_2 concentrations were measured using a Clark electrode connected to a Strathkelvin oxygen meter (model 781). Electrodes were calibrated with filtered seawater saturated with O_2 through aeration with a bubbling device for about 1 h. O_2 concentration was measured in control and experimental vessels before and after the 6-h incubation period. At the end of the incubation, O_2 concentrations in the experimental vessels were always within 70–96% of saturation levels. Respiration rates were derived from the difference in dissolved O_2 levels between control and experimental vessels. Rates of O_2 consumption were converted to energetic carbon requirements using the relationship $C = R(RQ)(12/22.4)$ where C is equivalent carbon respired (micrograms per individual per hour), R is respiratory rate (microlitres per individual per hour), RQ is respiratory quotient for protein (0.8), 12 is the atomic weight of carbon, and 22.4 is the molecular volume. Ingestion carbon equivalents were calculated assuming an average assimilation rate of 75% (Conover 1978).

Results

Trophic environment and distribution of *E. spinifera*

The surface distribution of Chl *a* from SeaWifs composite imagery for the cruise period (not shown) showed low values, especially in the regions outside the frontal systems. Values around the Subtropical Convergence and the Agulhas Front were in the range of 0.4–0.7 and 0.3–0.45 μg·L^{-1}, respectively. Vertical profiles of Chl *a* concentrations showed the consistent occurrence of a deep Chl *a* maximum positioned at about 30–60 m throughout the Subtropical Convergence – Agulhas Front area. Chl *a* levels within the deep Chl *a* maximum ranged from about 0.4–0.6 μg·L^{-1} at station 8 to 0.7–0.8 μg·L^{-1} at station 7 (Fig. 3). Size-fractionated data integrated for the water column show a clear dominance of the picophytoplankton size-class (<2 μm) throughout the survey region, accounting for 55–75 and 40–68% of the total Chl *a* at stations 8 and 7, respectively (Fig. 3). The 2- to 10-μm fraction contributed 12–38% and the >10-μm fraction 11–30% of the total stock. At the few stations where it was measured, the microphytoplankton size-class (>20 μm) exhibited extremely low values and contributed never more than 10% and generally less than 5% of the total.

Mesozooplankton biomass in the area ranged from 3.9 mg dry weight·m^{-3} (day) to 11.5 mg dry weight·m^{-3} (night) at station 7 and from 1.6 mg dry weight·m^{-3} (day) to 24.1 mg dry weight·m^{-3} (night) at station 8. The mesozooplankton assemblages were mainly copepods, euphausiids, and pteropods.

Along the cruise track, *E. spinifera* was collected only in the region north of the Subtropical Convergence at stations P3, G4, 7, and 8. Maximum concentrations were found at station 7, where on one occasion (10 February, 01:00 local time), levels of about 4 individuals·m^{-3} were recorded (Table 1). Average values obtained from double oblique hauls made with the Omori net were 1.13 ± 1.05 (SD) individuals·m^{-3} at station 7 and 0.022 ± 0.013 (SD) individual·m^{-3} at station 8. In terms of biomass, these are equivalent to 20.3 ± 28.03 (SD) and 0.19 ± 0.14 (SD) mg dry weight·m^{-3}, respectively. The only quantitative daytime tow was made at station G4, producing krill densities of 0.016 individual·m^{-3}, equivalent to a biomass of 0.23 mg dry weight·m^{-3} (Table 1). Both juveniles and subadults/adults were collected on most occasions.

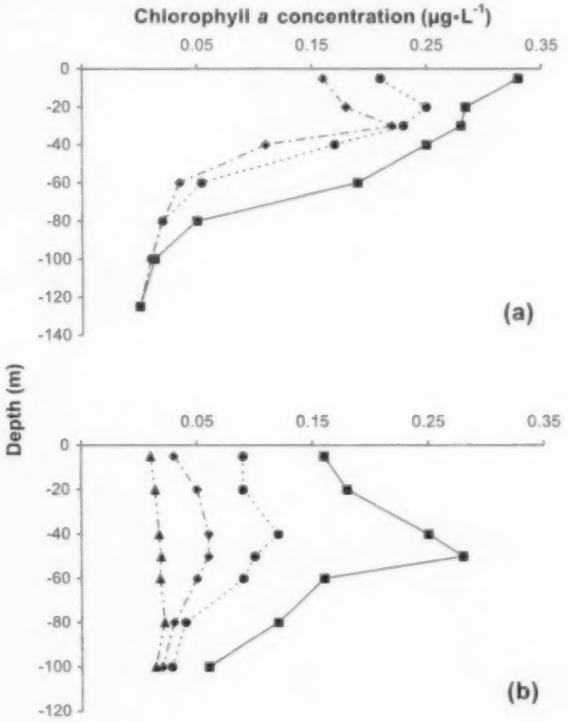

Fig. 3. Representative size-fractionated Chl *a* profiles from (*a*) station 7 (subtropical frontal region) and (*b*) station 8 (Agulhas frontal region) (courtesy of M. Fiala, Observatoire Océanologique, B.P. 44, 66651 Banyuls-sur-mer Cedex, France, unpublished data). ■, <2 μm; ●, 2–10 μm; ◆, 10–20 μm; ▲, >20 μm.

Table 1. Size structure, abundance, and biomass of *E. spinifera* from quantitative net tows made with a 1.6-m-diameter Omori net during the ANTARES-4 cruise.

Station	Local time	Depth (m)	Mean size (mm)	Abundance (individuals·m^{-3})	Biomass (mg·m^{-3})
G4	11:20	0–300	19.99	0.016	0.228
7	22:40	0–220	18.63	0.042	0.508
7	00:32	0–225	21.62	2.221	40.14
7	01:14	0–120	No data	4.150	No data
8	23:20	0–200	17.09	0.031	0.295
8	22:50	0–225	15.58	0.012	0.091

Note: Tow duration ranged from 30 min to 1 h.

Gut contents and grazing rates of *E. spinifera*

Gut content analysis showed that *E. spinifera* is able to ingest a large variety of prey. The most abundant items identified in the stomachs were centric diatoms, thecate flagellates, euphausiid eye cones, and crustacean appendages (Table 2). However, on average, the single greatest class of particles observed in the stomach contents was the amorphous material, not identifiable at the magnification used

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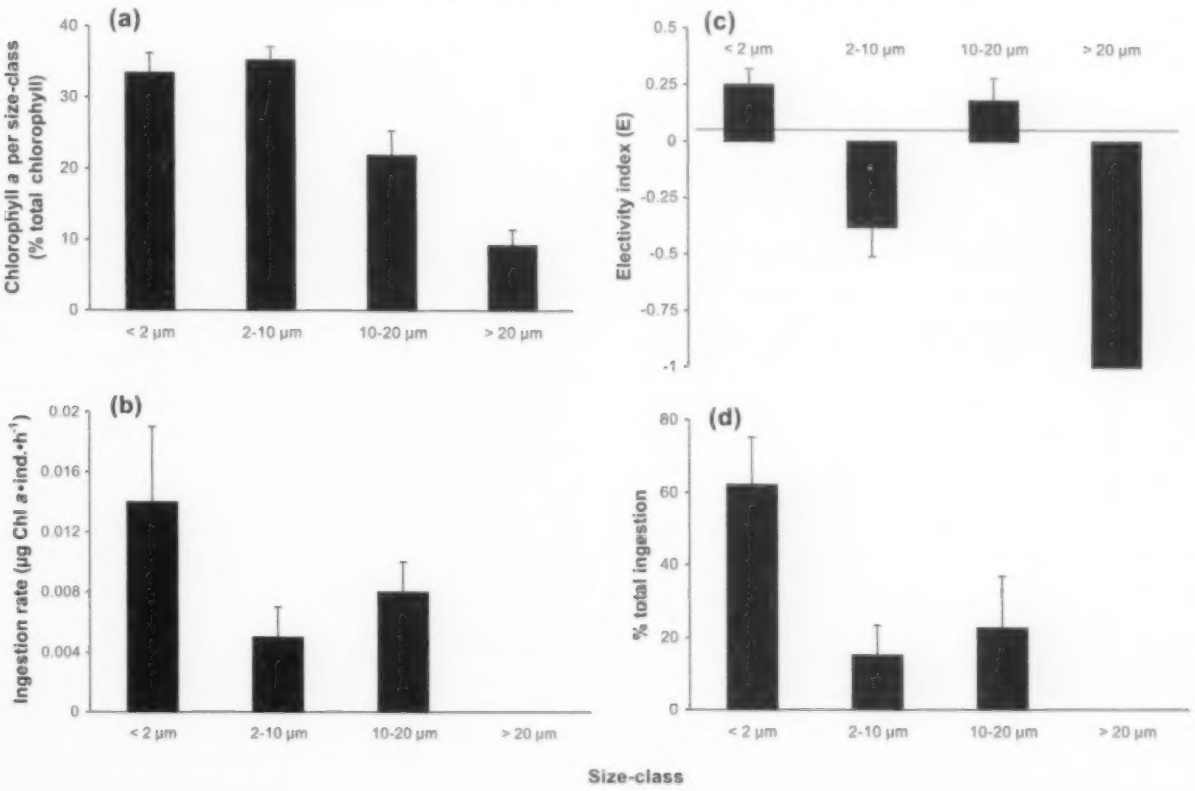
Table 2. Results of gut content analysis (mean number of items per individual gut ± SD) performed on 10 individual *E. spinifera* from the three main stations investigated during the ANTARES-4 cruise.

Food item	Size (µm)	Station G4, daytime tow	Station 7, nighttime tow	Station 8, nighttime tow
Amorphous material	*D* = 5–1500	81±5.7	59±20.8	70±18.3
Euphausiid eye cones	*L* = 37–43	0.2±0.6	37.9±73.5	30.6±67.2
Crustacean appendages	*L* = 600–1500	4.7±2.7	14.9±16.1	11.7±14.3
Tintinnids	*L* = 85–158	1.0±1.8	0±0	1.0±0.9
Globigerina sp.	*D* = 31–47	0.7±0.8	0.6±1.1	1.6±1.7
Thecate flagellates	*L* = 14–71	14.7±4.8	13.1±4.5	8.6±3.1
Centric diatoms	*D* = 12–27	54.5±14.0	53.3±26.8	27.9±11.8
Pennate diatoms	*L* = 35–88	1.0±0.8	0±0	0.2±0.4
Chain-forming diatoms	*D* = 6–9	3.7±8.4	9.8±22.1	0±0
Coccolithophorids	*D* = 12–36	1.1±1.0	2.3±6.3	0.5±1.0
Flagellate colonies (cells)	*D* = 22–47	0±0	20.5±23.7	0±0

Note: *D*, diameter; *L*, maximum length.

Fig. 4. (*a*) Size-fractionated Chl *a* standing stock, (*b* and *d*) ingestion rates, and (*c*) electivity index for *E. spinifera*.



(400×). The presence in the guts of many fresh cells from a variety of tissues associated with the fragments of euphausiids indicated that most individuals of *E. spinifera* may have been feeding in the net during capture.

Measured gut pigment contents ranged from 1.7 to 40.6 ng·individual^{-1} in subadult/adult and from 0.3 to 9.3 ng·individual^{-1} in juvenile *E. spinifera*. Highest levels were associated with the relatively high Chl *a* concentrations observed at the Subtropical Convergence (Fig. 3, station 7). Size-selectivity experiments with the autotrophic components of natural seawater showed that *E. spinifera* ingested only particles in the 0.7- to 20-µm range, virtually excluding the larger microphytoplankton. Within the 0.7- to 20-µm size range, 24% of the total ingestion comprised cells in the 10- to 20-µm range, 16% in the 2- to 10-µm range, and 60% in the <2-µm cells (Fig. 4).

Diel variations in gut pigment contents showed highest levels during late night (01:00–05:00 local time) and lowest

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Fig. 5. Diel variations in gut pigment contents in juvenile *E. spinifera* collected at station 8 (12–13 February 1999).

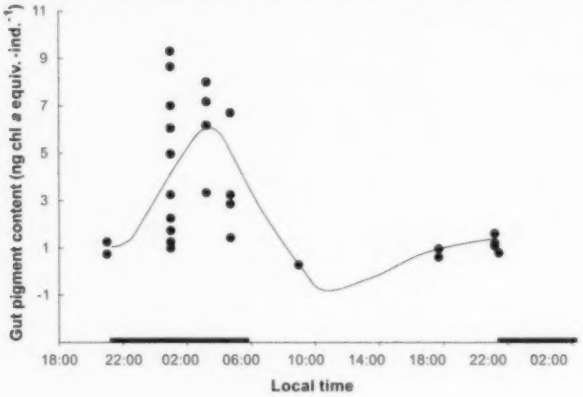
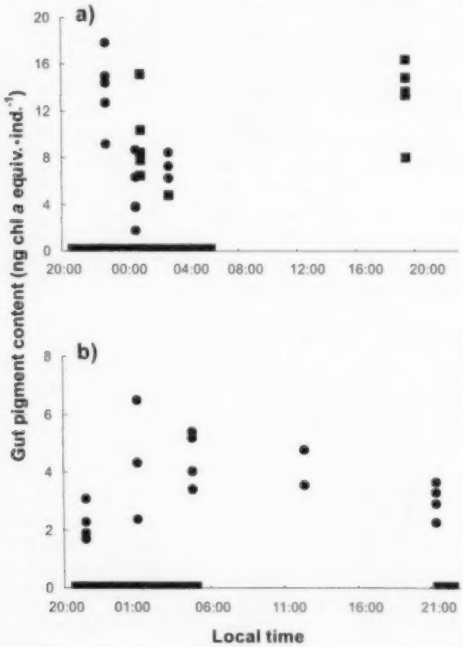


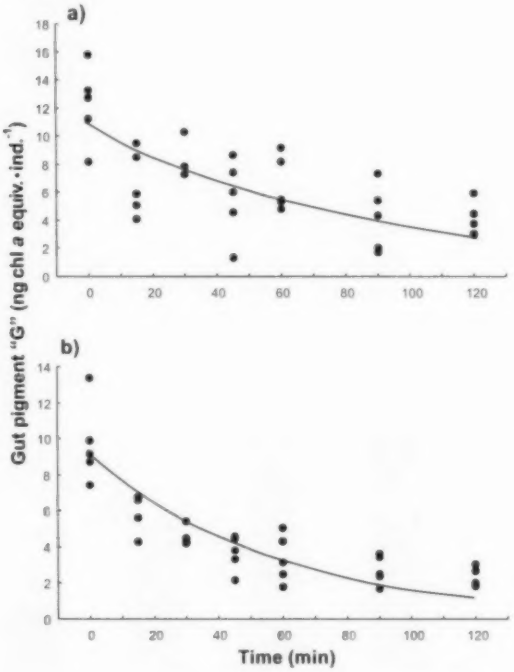
Fig. 6. Diel variation in gut pigment contents in subadult/adult *E. spinifera* collected at (*a*) station 7 (■, 5–6 February 1999; ●, 10 February 1999) and (*b*) station 8 (12–13 February 1999).



levels during daytime in juveniles (Fig. 5). However, given the limited data points available, this pattern is not recognizable in subadults/adults (Fig. 6). In both cases, variability in the gut pigment signal was much higher in nighttime samples than in daytime samples. Gut evacuation rates ranged from 0.59 to 0.96·h^{-1}, which corresponds to a gut turnover time of 1.04–1.68 h (Fig. 7). Gut pigment destruction levels were extremely high and ranged from 94 to 98% of the total pigment ingested (Table 3).

In situ grazing rates, corrected for the loss of pigments in the gut, ranged from 30.3 to 312 ng Chl *a* equiv.·individual^{-1}·h^{-1} at station 7 and from 47.6 to 183 ng Chl *a* equiv.·

Fig. 7. Gut evacuation rates (K) in subadult/adult *E. spinifera* collected at (*a*) station 7 (R^2 = 0.480, G_0 = 10.4 ± 0.8 (SD), K = 0.0099 ± 0.0019 (SD)·min^{-1}, 1/K = 1.68 h) and (*b*) station 8 (R^2 = 0.740, G_0 = 8.7 ± 0.5 (SD), K = 0.016 ± 0.002 (SD)·min^{-1}, 1/K = 1.04 h).



individual^{-1}·h^{-1} at station 8. These values are approximately one order of magnitude higher than those obtained from the size-selectivity experiments in vitro (Table 3).

Subadult/adult *E. spinifera* exhibited respiration rates of 3.65–20.5 µL O_2·individual^{-1}·h^{-1} (mean = 13.3 ± 5.44 (SD) µL O_2·individual^{-1}·h^{-1}, n = 10) during the cruise period (Table 4). Minimum energetic requirements calculated from these respiration rates were 1.56–8.79 µg C·individual^{-1}·h^{-1} (carbon equivalents) or 1.96–10.9 µg C·individual^{-1}·h^{-1} (ingestion equivalents) (Table 4).

Discussion

Euphausia spinifera is among the least studied species of krill, and virtually nothing is yet known about its biology and ecology. Our study has shown that the horizontal distribution of this species is typically patchy, while available data on its vertical distribution are not sufficient to draw conclusions on possible diel migration patterns. The dense concentrations found in some nighttime samples (01:00 local time) collected with the Omori net at station 7 (>4 individuals·m^{-3}) indicate that the species exhibits swarming behaviour similar to that observed in many other euphausiids.

Individuals of *E. spinifera* collected during the early part of the survey exhibited green stomachs, with abundant autotrophic material in their guts. The contribution of algal material to the energy budget of this species was estimated using a combination of ecophysiological parameters, including gut pigment content, gut evacuation rate, and gut pigment de-

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Table 3. Chl *a* concentration, ingestion rates, gut pigment content, and pigment destruction efficiency for *E. spinifera* during the ANTARES-4 cruise.

Experiment	Individual	Chl *a* concentration ($\mu g \cdot L^{-1}$)	Ingestion rate (ng pigment·individual^{-1}·h^{-1})	Gut pigment content (ng·individual^{-1})	% pigment destruction
1	1	0.33	7.75	0.38	95.1
1	2	0.31	3.87	0.23	94.2
1	3	0.32	6.21	0.32	94.8
1	4	0.33	15.51	0.38	97.6
2	1	0.32	11.95	0.34	97.2
2	2	0.34	19.93	0.38	98.1
2	3	0.37	17.27	0.48	97.2
2	4	0.37	19.11	0.29	98.5

Table 4. Respiration rates and metabolic carbon requirements of *E. spinifera* (station 7, night) during the ANTARES-4 cruise.

Date	Incubation time (h)	Respiration rate ($\mu L\ O_2$·individual^{-1}·h^{-1})	Carbon equivalents (μg C·individual^{-1}·h^{-1})	Ingestion equivalents (μg C·individual^{-1}·h^{-1})
7 Feb. 1999	6.75	11.01	4.72	6.29
7 Feb. 1999	7.13	3.65	1.56	2.08
10 Feb. 1999	6.87	14.02	6.01	8.01
10 Feb. 1999	6.33	20.52	8.79	11.72
10 Feb. 1999	6.74	14.85	6.36	8.48
10 Feb. 1999	5.97	18.07	7.74	10.32
10 Feb. 1999	6.25	4.61	1.98	2.64
10 Feb. 1999	5.80	16.22	6.95	9.27
10 Feb. 1999	7.09	14.44	6.19	8.25
10 Feb. 1999	6.83	15.46	6.63	8.84

struction. While the main objective of measuring these parameters was to estimate in situ grazing rates on phytoplankton, they also revealed some interesting feeding characteristics that are worth comparing with those of similar, better known euphausiid species from the Southern Ocean.

For instance, diel variations in gut pigment contents of *E. spinifera* showed highest levels during late night (01:00–05:00 local time) and lowest levels during the day in both juveniles and subadults/adults. The variability in the gut pigment signal was also much higher in nighttime samples than in daytime samples in both cases. This feature has not been reported previously in euphausiids (Mauchline 1980; Miller and Hampton 1989; Perissinotto et al. 1997) and suggests either a different degree of herbivory in the various animals collected during their most active feeding period or a different rate of feeding in different individuals, with only part of the population feeding at maximum rate at any given time.

Euphausia spinifera also exhibits the highest gut pigment destruction rates among the major krill species of the Southern Ocean, with an average of about 97% of the total autotrophic material being rapidly converted to nonfluorescent end products. Average values for *Euphausia superba* range from 84% to 81% (Pakhomov et al. 1997), while for *Euphausia vallentini*, pigment destruction rates are about 55% (Perissinotto 1992). It is difficult, however, to relate this response to any difference in the feeding dynamics of the three species, since the feeding history of each individual also plays an important role in controlling the extent of pigment degradation (Mayzaud and Razouls 1992; Tirelli and Mayzaud 1998).

The same applies to the estimates of gut evacuation rate in the three species, with *E. spinifera* exhibiting values of 0.59–0.96·h^{-1} compared with 0.10–0.31·h^{-1} for *E. superba* (Perissinotto et al. 1997) and 0.59–0.81·h^{-1} for *E. vallentini* (Perissinotto 1992). It is well known that gut evacuation rate varies widely with temperature (Kiørboe et.al. 1982; Dam and Peterson 1988), food concentration (Dagg and Walser 1987), and food quality (Mayzaud et al. 1998). These intraspecific effects will, therefore, interfere with real interspecific variations related to differences in the ecophysiology of the various species.

Euphausia spinifera size-selectivity experiments showed a lack of any grazing on naturally occurring phytoplankton cells larger than 20 µm. There was a positive ingestion of particles ranging from <2 to 20 µm and selectivity for two size groups, i.e., <2 and 10–20 µm. Despite the high abundance of the 2- to 10-µm size-class, *E. spinifera* appears to select negatively for this size group. This could tentatively be explained in terms of metabolic requirements of the animal in relation to the nutritional content of the various components of the different size-classes. A comparison with some of the most important krill species of the Southern Ocean shows that the capability of *E. spinifera* to select autotrophic food particles according to size is very reminiscent of the pattern observed in *E. similis*, which is widespread both in the subantarctic and in the subtropical regions (Fig. 8). Both species seem to actively exclude phytoplankton larger than 20 µm and smaller than 5–10 µm while ingesting cells mainly in the 10- to 20-µm range. However, a major difference between the two species is the high electivity index of *E. spinifera* for the <2-µm size class, while *E. similis* appears to select slightly negatively for this

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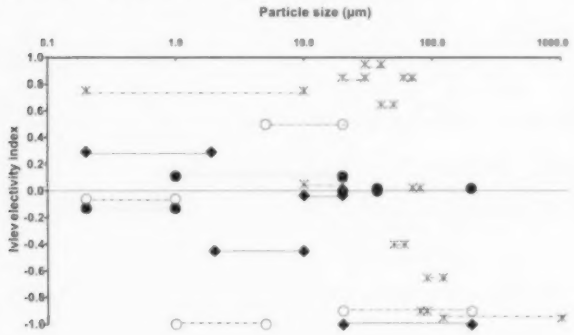
Fig. 8. Comparison of the Ivlev electivity indexes (E) for *E. spinifera* and for other major krill species occurring in the Southern Ocean ($E = (r - p)/(r + p)$, where r is the percentage of food of a given size-class ingested and p is the percentage of this size-class available in the water (Poulet 1973)). Data for *E. similis* and *E. vallentini* are from Perissinotto (1992) and data for *E. superba* are from Opalinski et al. (1997). ◆, *E. spinifera*; ○, *E. similis*; ●, *E. vallentini*; *, *E. superba*.



group. The Antarctic species *E. superba*, on the other hand, exhibits a positive selection for most particle sizes from <10 to 70–80 µm and then heavily excluding all particles larger than 80 µm. The subantarctic species *E. vallentini* shows clear evidence of positive selection for 1- to 20-µm particles but negative selection for particles smaller than 1 µm (Fig. 8). Apart from the obvious size differences in the average length and mass of these species, it is possible that these patterns reflect the general prevalence of the picophytoplankton size-class in the subtropical region, of nanophytoplankton in the subantarctic region, and of nano- and microphytoplankton in the high Antarctic (Weber and El Sayed 1987; Laubscher et al.1993).

To what extent, however, is the autotrophic component of the diet of *E. spinifera* providing a major proportion of its total food demands? An approximate estimation of its individual daily ration can be obtained from the general relationship of carbon content (C) versus dry weight (DW) of Ikeda and Bruce (1986): C = 42.6–47.1% DW. This shows that the average carbon content of *E. spinifera* collected during the ANTARES-4 survey was 5.25 ± 1.79 (SD) mg C·individual^{-1}, with a range of 3.15–7.70 mg C·individual^{-1}. Using the daily ingestion rates obtained in situ, the corresponding carbon ration would be in the range of 3.06% (station 7) to 4.11% (station 8). Now, while these figures are not very low compared with the experimental results obtained for other euphausiid species, they are nevertheless hardly sufficient to account for the basic energetic requirements associated with respiration.

Indeed, converting the carbon ingestion equivalents of Table 4 into percent body carbon required per day, a range of 0.90–4.99% is obtained. Since it is generally accepted that total energetic demands, including the portions devoted to growth, molting, and reproduction, are some 5–10 times higher than those measured for respiration alone (Clarke et al. 1988; Daly 1990; Atkinson and Snÿder 1997; Perissinotto et al. 1997), it is clear that the autrophic component of the diet of *E. spinifera* during the time of the survey needed to be complemented with large amounts of heterotrophic material.

This is confirmed by results of the gut content analysis, which show that small flagellates, tintinnids, foraminiferans, and crustaceans formed at times a very important part, if not the bulk, of the total material ingested. Crustacean fragments were particularly abundant in the guts of nighttime samples and were represented mainly by clusters of euphausiid eye cones, pleopods, and thoracic legs. Many pieces of tissue associated with these fragments exhibited well-structured and perfectly preserved cells that had clearly been ingested just before the death of their predators. It seems very possible that this may be due to net feeding during capture, possibly in the cod-end. It is also worth noting that despite the many crustacean fragments encountered in the analysis, none could be conclusively attributed to copepod species, despite the numerical dominance of this group in the net samples collected at both stations 7 and 8. Although *E. spinifera* is clearly capable of ingesting heterotrophic organisms, often of large size and in great amounts, it remains to be seen whether this indeed reflects its average feeding pattern under natural conditions.

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Density and size of juvenile salmonids in response to placement of large woody debris in western Oregon and Washington streams

Philip Roni and Thomas P. Quinn

Abstract: Thirty streams in western Oregon and Washington were sampled to determine the responses of juvenile salmonid populations to artificial large woody debris (LWD) placement. Total pool area, pool number, LWD loading, and LWD forming pools were higher in treatment (LWD placement) than paired reference reaches during summer or winter. Juvenile coho salmon (*Oncorhynchus kisutch*) densities were 1.8 and 3.2 times higher in treated reaches compared with reference reaches during summer and winter, respectively. The response (treatment minus reference) of coho density to LWD placement was correlated with the number of pieces of LWD forming pools during summer and total pool area during winter. Densities of age-1+ cutthroat trout (*Oncorhynchus clarki*) and steelhead trout (*Oncorhynchus mykiss*) did not differ between treatment and reference reaches during summer but were 1.7 times higher in treatment reaches during winter. Age-1+ steelhead density response to treatment during summer was negatively correlated with increases in pool area. Trout fry densities did not differ between reaches, but the response of trout fry to treatment was negatively correlated with pool area during winter. Our research indicates that LWD placement can lead to higher densities of juvenile coho during summer and winter and cutthroat and steelhead during winter.

Résumé : Des échantillonnages dans trente cours d'eau de l'ouest de l'Oregon et du Washington ont permis d'étudier les réactions des populations de jeunes saumons à l'introduction artificielle de débris ligneux de grande taille (LWD) dans le lit du cours d'eau. La surface et le nombre de fosses, la charge de LWD, et l'incidence de fosses formées par la présence de LWD sont toutes plus élevés dans les zones expérimentales dans lesquelles on a ajouté des LWD, que dans les zones témoins appariées, tant en hiver qu'en été. Les densités des jeunes saumons coho (*Onchorhynchus kisutch*) sont 1,8 fois plus grandes que dans les zones témoins durant l'été et 3,2 fois plus élevées en hiver. La modification de la densité des saumons (densité expérimentale moins densité de la zone témoin) à la suite de l'introduction de LWD est reliée en été au nombre de pièces de LWD qui entraînent la formation de fosses et, en hiver, à la surface totale des fosses. Les densités de la truite fardée (*Oncorhynchus clarki*) et de la truite arc-en-ciel anadrome (*Oncorhynchus mykiss*) d'âge 1+ ne varient pas entre les zones expérimentales et les zones témoins en été, mais sont 1,7 fois plus élevées en hiver dans les zones expérimentales. La densité des truites arc-en-ciel d'âge 1+ en été est en corrélation négative avec l'augmentation de la surface des fosses. Les densités des alevins de la truite fardée ne varient pas d'une section à une autre, mais elles sont en relation négative avec la surface des fosses en hiver. Notre étude montre que l'addition de LWD peut conduire à des densités accrues de saumons coho juvéniles tant en été qu'en hiver, de même que des truites fardées et des truites arc-en-ciel en hiver.

[Traduit par la Rédaction]

Introduction

The factors controlling the populations of salmonid fishes are numerous and complex, but it is widely believed that instream habitat plays a role in population density, at least for stream-rearing species (National Research Council 1996). In an effort to mitigate for degradation and loss of fish habitat from anthropogenic disturbance and stop or reverse the declines in salmonid populations in recent years, stream restoration projects have become common in the Pacific Northwest. The placement of boulders, logs, and woody debris directly into the stream channel to create pools, provide cover, and reduce gravel movement is an integral part of most of these stream restoration projects and recovery efforts for Pacific salmon (Rodgers et al. 1992; Chapman 1996). Unfortunately, the research and monitoring that has occurred has often been inadequate to determine the effectiveness of various stream restoration activities at increasing fish abundance (Reeves et al. 1991*b*; Chapman 1996).

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The restoration and enhancement of stream fish habitat has been occurring for more than 60 years in the United States (Reeves et al. 1991*a*). Stream restoration techniques were originally pioneered in the midwestern United States but have been modified for use in the steeper, high-energy streams common in western North America (Reeves et al. 1991*a*). The interest in habitat restoration in the Pacific Northwest has increased dramatically since the early 1980s, when the importance of woody debris in maintaining and creating fish habitat became widely accepted (Bisson et al. 1987). Prior to this time, "stream cleaning" (removal of wood from streams) was a common practice (Bisson et al. 1987). In the last 20 years, many studies have emphasized the critical role that large woody debris (LWD) plays in creating and maintaining fish habitat in streams (National Research Council 1996). In-stream LWD can create pools, increase habitat complexity, reduce sediment transport, trap gravel needed for spawning, stabilize stream channels, provide food for aquatic invertebrates, and provide stream nutrients, increasing overall stream productivity (Bisson et al. 1987). Consequently, in-stream LWD placement has become one of the most common techniques for improving fish habitat in an effort to compensate for the reductions in LWD caused by stream cleaning and various land use practices (Kauffman et al. 1997).

More than a decade ago, Reeves and Roelofs (1982) identified the need for better evaluation of habitat improvement projects. More recently, Reeves et al. (1991*a*), Chapman (1996), and Kauffman et al. (1997) reiterated the need for comprehensive evaluation of in-stream restoration efforts. Monitoring of stream restoration projects in the western United States and Canada has focused primarily on the physical habitat responses and whether LWD structures functioned as designed rather than on biotic responses. Many studies have documented increases in pool frequency, pool depth, woody debris, and sediment retention following placement of in-stream structures (e.g., House et al. 1991; Riley and Fausch 1995; Cederholm et al. 1997). However, the extent to which the structures remain in place and functioning after several years is less clear (Frissell and Nawa 1992; Roper et al. 1998).

Biological evaluations of in-stream restoration have also produced highly variable results. There have been a number of encouraging reports of increased densities of salmonids following restoration efforts. House et al. (1989) and House (1996) reported increased juvenile coho salmon (*Oncorhynchus kisutch*) densities in several Oregon coastal streams, with small increases in densities of juvenile cutthroat trout (*Oncorhynchus clarki*) and steelhead trout (*Oncorhynchus mykiss*). Cederholm et al. (1997) reported significantly more coho salmon smolts following restoration in a Washington stream. On the other hand, numerous authors have reported no significant biological response or even decreases in salmonid abundance following restoration. Reeves et al. (1997) reported no significant difference in coho parr or smolt numbers following restoration. Beschta et al. (1994) and Chapman (1996) reviewed several case studies on restoration efforts in the Columbia Basin and western United States and found little evidence of increased fish numbers. The inconsistent results of these studies further emphasize the need for continued biological evaluation of in-stream restoration efforts and the need for a multispecies approach.

Our research was developed to provide a broad-scale physical and biological evaluation of in-stream LWD placement efforts (restoration) in western Oregon and Washington. The overall objectives were to determine whether the artificial placement of LWD produces a significant change in physical habitat and juvenile salmonid abundance. Specifically, we tested the null hypotheses that paired treatment and reference reaches would not differ in (*i*) densities of woody debris and pool area or (*ii*) densities of juvenile coho salmon and cutthroat and steelhead trout in summer and winter and that (*iii*) the magnitude of fish response to treatment would not depend on the magnitude of change in habitat and (*iv*) the sizes of the fish would not differ between treatment and reference reaches.

Materials and methods

We used the extensive posttreatment design (Hicks et al. 1991) to determine the response of juvenile salmonids to artificially placed LWD. This design involves comparison between treatment and reference reaches at a large number of sites after restoration efforts, in contrast with pre- and post-treatment comparisons and comparisons between paired treatment and control streams. The extensive posttreatment design has frequently been used to assess the impacts of forestry and other land use practices on salmonids and their habitats and is particularly well suited to reach-scale habitat restoration projects (Hicks et al. 1991).

Thirty streams in western Oregon and Washington (Fig. 1) with paired treatment and reference (control) reaches were sampled during both summer and winter between August 1996 and April 1999. Treatment was defined as the artificial placement of LWD within the active stream channel. Paired treatment and reference reaches 75–120 m long were selected in each stream. Study reaches were at least 10 times bankfull width in length, and most reaches were 100 m long. Each stream was surveyed once in summer (August–September) and winter (January–March) and reaches within a stream were sampled on the same day. Only those sites that had treatment and reference reaches of similar gradient, confinement, and channel width were selected.

The selection of study streams with paired treatment and reference reaches was based in part on physical and biological stream characteristics including stream size, bankfull width, channel type, and fish species composition. The selection of similar treatment and reference reaches is critical for a study of this nature, and it is important to control "background" features not influenced by LWD placement. In particular, reaches within a stream need to be of similar slope, width, discharge, and length. These features can affect the fish community and also influence the action of LWD. The placement of LWD can alter the number, area, and size of pools and the substrate volume and size (Bisson et al. 1987; Reeves et al. 1991*b*; Cederholm et al. 1997). Therefore, within a given stream, we selected treatment and reference reaches of similar slope (paired t test, $p = 0.46$) and channel width (paired t test, $p = 0.13$) (Table 1). We fixed the lengths of the reaches to be identical, and the proximity of the reaches insured that the discharges were essentially identical, although the distribution of point velocities might differ. More than 100 LWD placement projects were examined in western Washington and Oregon, but only 30 had suitable treatment and reference reaches with similar flow, channel width, gradient, confinement, and riparian vegetation. At nine sites, the biologist responsible for constructing the project selected treatment and reference reaches prior to treatment, and we used these as our treatment and reference reaches when possible. Only projects in

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Fig. 1. Map of the 30 study streams in western Oregon and Washington, U.S.A.

which the artificially placed LWD remained in the channel after several high-water events, usually over several winters, were included. Reference reaches were located 200 m or more upstream from treatment reaches.

The study streams ranged from 4 to 12 m in bankfull width and from 0.5 to 4.2% slope (Table 1). The age of the restoration projects (date of last LWD placement to date of sampling) ranged from 1 to 10 years. Annual precipitation varied from and 107 to 315 cm. Dominant forest types were primarily Douglas-fir (*Pseudotsuga menziesii*), Sitka spruce (*Picea sichensis*), and western hemlock (*Tsuga heterophylla*). The dominant drainage geology was volcanic, sedimentary, or glacial–alluvial and varied by site but was consistent for reaches within a stream. The elevations of the study sites ranged from 12 to 789 m and drainage area upstream of our study reaches ranged from 124 to 2388 ha (Table 1).

Habitat within each stream reach was classified using a modification of the methods and habitat types described by Bisson et al. (1982). Total surface area of each habitat was estimated by measuring the total habitat length and multiplying by the average of three to five width measurements. The gradient of each reach and individual habitat unit was measured using a hand level, survey (stadia) rod, and tape measure. Habitat-specific stream slope (gradient) was used to distinguish between riffles and cascades. Discharge was estimated with a flowmeter prior to completion of each survey.

All natural and artificially placed LWD within the active channel greater than 10 cm in diameter and 1.5 m long was inventoried. The diameter class (small, 10–20 cm; medium, 20–50 cm; large, >50 cm) and approximate length were recorded. The function of an individual piece of LWD based on its influence on pool formation and channel scour was classified into one of three categories: (*i*) dominant — primary factor contributing to pool formation, (*ii*) secondary — influences zone of channel scour but not responsible for pool formation, and (*iii*) negligible — may provide cover but not involved in scour (Montgomery et al. 1995). Preproject maps and the presence of anchoring material (cable, nonnative boulders, or rebar) or aluminum tags (placed on wood during construction) were used to assist in classifying LWD as artificially placed or natural.

Multiple-removal electrofishing was used in summer to estimate fish abundance within each individual habitat (Carle and Strub 1978). Each habitat was sampled separately by placing 3.2-mm-mesh blocknets at the upstream and downstream boundaries of each habitat unit to prevent immigration or emigration during sampling. Three removals were made through each habitat and a fourth was made if a 50% or greater reduction in fish numbers was not seen between the second and third passes. Each electrofishing removal consisted of one upstream and one downstream pass using a pulsed DC electrofisher. All fish captured were anesthetized with tricaine methanesulfonate (MS 222), identified, measured to the nearest millimetre, and then released. Based on length–frequency distributions, all steelhead or cutthroat trout greater than 60 mm during summer or 80 mm during winter were considered age 1+. All trout less than these lengths were considered fry (age 0 or young-of-year). Such fry could not be reliably identified to species and so were simply called trout.

High flows during winter months often precluded the use of multiple-removal electrofishing, so night snorkel surveys were used to estimate juvenile salmonid abundance during winter. Roni and Fayram (2000) demonstrated that winter night snorkeling was nearly as accurate as multiple-removal electrofishing and suitable for a wider range of conditions. Juvenile salmonids generally emerge from concealment 30–60 min after sunset at temperatures below 8–9°C (Roni and Fayram 2000). Therefore, snorkeling began at least 1 h after sunset, and only on nights with either complete cloud cover or no visible moonlight to assure that natural light levels were consistently low during night snorkel surveys. Surveys were conducted in 1997 during February and March in Washington and in 1998 from January to mid-March in Oregon to assure that sampling occurred prior to the outmigration of salmonid smolts.

One diver entered the habitat from the downstream end and slowly moved upstream, stopping occasionally to relay the number, sizes, and species of fish observed to a second individual on the bank. In streams greater than 10 m wide, two snorkelers worked side by side to cover the entire width of the stream. A halogen dive light was used to illuminate areas and identify fish. Fish length was visually estimated to the nearest 10 mm using a ruler attached to the diver's glove. Water temperature and flow were measured downstream of each site before electrofishing and snorkeling. Discharge and temperature among streams ranged from 0.10 to 0.15 $m^3 \cdot s^{-1}$ and from 7.5 to 16.9°C during electrofishing surveys and from 0.14 to 2.1 $m^3 \cdot s^{-1}$ and from 2.5 to 8.5°C during snorkel surveys.

Differences in habitat, LWD, and fish abundance between treatment and reference reaches were compared using paired *t* tests. A Bonferroni correction was applied to compensate for the increased likelihood of finding a significant difference when performing multiple *t* tests. A family alpha level of 0.05 was used to determine significance and divided by the number of tests performed on each separate data set (fish, habitat, and LWD). This resulted in a significance level of 0.01 for each individual *t* test. A $\log_{10}(x + 1)$ transformation was used on fish data to meet the basic assumptions of a *t* test (additive data, normal distribution, equal variances) and ac-

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Table 1. Physical characteristics of study streams measured during summer.

Stream	Dominant geology	Elevation (m)	Drainage area (ha)	Precipitation (cm·year^{-1})	Bankfull width (m)	Slope (%)		Pool area (%)	
						R	T	R	T
Oregon									
Bear	Volcanics	244	1580	320	10.1	1.2	1.5	0.29	0.79
Bergsvik	Sedimentary	122	540	308	9.2	1.0	0.9	0.81	0.76
Bewley	Sedimentary	12	639	235	6.8	0.5	1.1	0.55	0.62
Buster	Sedimentary	232	1627	228	7.8	0.8	0.5	0.89	0.79
Deer	Sedimentary	219	414	169	4.6	0.4	1.2	0.86	0.90
Elliott	Volcanics	427	720	236	11.0	1.4	2.4	0.55	0.55
Farmer	Sedimentary	73	727	260	7.3	1.8	1.6	0.34	0.42
Kenusky	Volcanics	207	1158	167	6.4	1.5	1.2	0.65	0.64
Killam	Volcanics	110	863	298	9.3	3.2	3.0	0.37	0.49
Klootchie	Sedimentary	61	1011	299	8.9	2.2	1.9	0.36	0.64
Lobster	Sedimentary	207	1254	233	9.9	1.8	1.7	0.44	0.65
Louisignont	Volcanics	244	1715	201	9.6	0.8	0.6	0.78	0.85
Ltl. Nestucca (South Fork)	Sedimentary	122	981	250	9.6	0.9	1.6	0.25	0.77
Rock (North Fork)	Volcanics	390	1893	286	9.9	1.3	0.7	0.25	0.52
Tobe	Volcanics	165	680	236	5.9	2.5	2.8	0.38	0.51
Washington									
Beaver	Glacial–fluvial	233	124	189	5.5	1.8	2.3	0.72	0.74
Benson	Sedimentary	320	459	217	11.6	1.8	1.9	0.35	0.31
Burn	Sedimentary	481	733	227	6.4	2.2	2.0	0.55	0.69
French	Igneous	172	1783	213	16.5	2.3	2.2	0.18	0.25
Harris	Volcanics	292	311	354	7.2	1.1	1.0	0.29	0.66
Hoppers	Volcanics	73	467	269	4.2	0.8	0.7	0.78	0.96
Hyas	Sedimentary	121	2000	290	12.2	1.3	0.7	0.36	0.59
Laughing Jacobs	Glacial–fluvial	23	335	119	6.8	2.5	2.3	0.19	0.38
Midnight	Igneous	598	567	212	5.6	3.9	4.5	0.31	0.34
Newbury	Volcanics	170	302	317	5.8	1.8	1.9	0.45	0.46
Porter	Volcanics	122	2388	170	10.0	1.3	2.3	0.56	0.67
Punch	Sedimentary	110	271	353	8.5	3.6	3.2	0.53	0.44
Shuwah	Sedimentary	197	305	297	6.5	1.4	1.9	0.56	0.80
Soosette	Glacial–fluvial	45	1225	108	11.1	1.7	1.7	0.19	0.29
Townsend	Volcanics	789	809	199	4.3	3.9	3.1	0.33	0.60

Note: Drainage area (area) for a given stream was calculated as total drainage area upstream of the restoration site. Geology and elevation are from unpublished U.S. Geologic Survey data. R, reference; T, treatment.

count for any zero or low counts. Multiple regression was used to examine the relationship(s) between fish response (ratio of treatment density to reference density) and difference in physical variables including pool area, percent pool area, riffle area, pieces of LWD, pieces of LWD creating pools (functional LWD), number of habitats, channel slope, geographic region (Washington or Oregon), and structure type (engineered or naturally placed log). All ratios of treatment to reference (pool area, pieces of LWD, etc.) were also log transformed ($\log_{10}(x)$) to meet the assumptions of a t test (additive data, normal distribution, equality of variances). The average ratio of fish densities (treatment over reference) was calculated as a geometric mean. This was necessary to compensate for differences in fish densities among streams and to assure that all streams were given equal weight.

Results

Physical habitat

Treatment and reference reaches had identical lengths and were similar in slope and bankfull width. However, they differed in physical habitat features expected to respond to LWD placement. The total number of pieces of LWD per 100 m was significantly higher in treatment than in reference reaches during both summer (20–80 versus 8–63, $p < 0.01$) and winter (16–78 versus 4–64, $p < 0.01$) (Table 2) and averaged 1.83 and 1.89 times greater in treatment reaches in summer and winter, respectively. The total number of pieces of functional LWD was also significantly higher in treatment than in reference reaches during both summer and winter ($p < 0.01$) and averaged 2.83 and 2.96 times greater in treatment than in reference reaches. The difference in functioning LWD (LWD creating pools) between treatment and reference reaches was correlated with the difference in pool area ($p = 0.02$, $r^2 = 0.18$).

Treated stream reaches exceeded reference reaches in total wetted area, total number of habitat units, total pool area, and total number of pools during both summer and winter ($p < 0.01$ in all cases). However, total riffle area was not significantly different between treatment and reference reaches during either summer or winter ($p = 0.05$ and 0.09, respectively). Total number of habitat units (an indicator of habitat complexity) was not significantly different between treatment and reference reaches during summer ($p = 0.05$) but

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Table 2. Number of pieces (per 100 m) of artificially placed, functioning (creating pools, summer only), and total LWD in reference (R) and treatment (T) reaches for each stream.

Stream	Project age	No. of pieces placed LWD (T only)	Functioning LWD		Total LWD			
					Summer		Winter	
			R	T	R	T	R	T
Oregon								
Bear	4	11	1	5	9	40	21	54
Bergsvik	3	3	0	5	8	40	14	55
Bewley	3	6	0	2	11	35	10	16
Buster	2	14	2	3	41	27	25	24
Deer	3	6	5	4	34	31	29	24
Elliott	1	12	2	1	29	57	26	57
Farmer	3	13	0	5	41	46	26	37
Kenusky	3	6	0	3	26	66	29	73
Killam	3	8	1	1	17	39	14	40
Klootchie	3	8	0	3	27	50	14	48
Lobster	11	6	0	6	40	70	42	43
Louisignont	2	8	2	6	18	28	15	50
Ltl. Nestucca (South Fork)	3	10	0	6	11	39	12	47
Rock (North Fork)	3	7	1	4	27	48	43	55
Tobe	4	10	0	8	28	52	22	42
Washington								
Beaver	3	11	7	8	25	54	31	73
Benson	7	18	2	6	18	40	42	52
Burn	5	15	2	8	35	80	25	66
French	6	42	2	8	23	55	20	66
Harris	12	12	0	9	22	24	22	27
Hoppers	1	10	5	10	33	35	43	46
Hyas	6	16	0	5	0	42	4	50
Laughing Jacobs	2	35	2	5	61	66	49	53
Midnight	4	24	1	9	27	28	31	30
Newbury	12	9	1	4	9	20	15	24
Porter	5	22	2	4	25	62	21	55
Punch	12	9	8	9	63	59	64	78
Shuwah	1	12	6	9	38	47	52	53
Soosette	3	28	1	2	9	48	16	72
Townsend	2	17	3	7	29	42	37	51

Note: LWD, large woody debris. Project age represents the number of years between LWD placement and our surveys.

was significantly higher in treatment reaches during winter ($p < 0.01$). Pool area in treatment reaches averaged 1.52 times that in reference reaches during summer and 1.51 during winter, and total wetted area increased by a factor of 1.11 in summer and 1.08 in winter. Treated reaches had 1.31 times more pools than reference reaches in summer and 1.48 times more in winter. The total number of habitat units was 1.11 and 1.22 times higher in treatment than in reference reaches during summer and winter, respectively.

Salmonid densities

Summer

Juvenile coho salmon densities (fish per metre) in summer were higher in treatment than in reference reaches (1.81 times higher, $p < 0.01$), but densities of age-1+ cutthroat, age-1+ steelhead trout, and trout fry did not differ ($p = 0.08$, 0.45, and 0.24, respectively) (Table 3). There was a positive linear relationship between the response of summer coho salmon densities (treatment to reference ratio) and the number of pieces of functioning LWD ($p < 0.01$, $r^2 = 0.25$) (Fig. 2). No significant relationship was detected between coho response and any other individual or combination of physical variables ($p > 0.10$). No significant relationship existed between any individual or combination of physical variables and cutthroat trout response to treatment ($p > 0.15$). Age-1+ steelhead trout densities were negatively correlated with a difference in pool area ($p = 0.01$, $r^2 = 0.32$) and percent pool area ($p < 0.01$, $r^2 = 0.45$ (Fig. 2)) and positively correlated with a difference in riffle area ($p < 0.01$) but not with any other combination or individual physical variables. No response was detected between trout fry and any physical variables or combination of physical variables ($p > 0.50$ for all models) during summer.

Winter

Coho salmon densities were 3.23 times higher in treatment stream reaches ($p < 0.01$) and age-1+ cutthroat and steelhead trout densities were 1.70 and 1.73 times higher, respectively, during winter ($p < 0.01$) (Table 3). Trout fry den-

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Table 3. Ratio (geometric mean) of salmonid densities for treatment to reference reaches for all 30 sites combined and separated by state.

Species	Oregon	Washington	All sites
Summer			
Coho salmon	2.08*	1.55	1.81*
Cutthroat trout (age 1+)	1.10	1.55	1.27
Steelhead trout (age 1+)	1.03	1.37	1.19
Trout fry	1.31	1.05	1.21
Winter			
Coho salmon	4.25*	2.33*	3.23*
Cutthroat trout (age 1+)	1.90*	1.44	1.70*
Steelhead trout (age 1+)	1.82*	1.48	1.73*
Trout fry	1.25	1.24	1.25

*Significant difference ($p < 0.05$).

sities did not differ between treatment and reference reaches ($p = 0.24$). Multiple regression analysis indicated that coho response during winter was significantly correlated with pool area and restoration type (engineered or natural) ($p < 0.01$, $r^2 = 0.38$) but not with any other physical variables either in combination or individually ($p > 0.10$). Pool area alone explained 27% of the variation in coho salmon response to restoration among sites ($p < 0.01$) (Fig. 3*a*). No relationships were detected between age-1+ cutthroat trout winter response to treatment and any combination of variables or single physical variable ($p > 0.36$). Age-1+ steelhead response was not correlated with any combination of variables or single physical variable ($p > 0.10$). Trout fry response to treatment was negatively correlated with difference in percent pool area ($p = 0.04$, $r^2 = 0.20$) (Fig. 3*b*).

Fish length

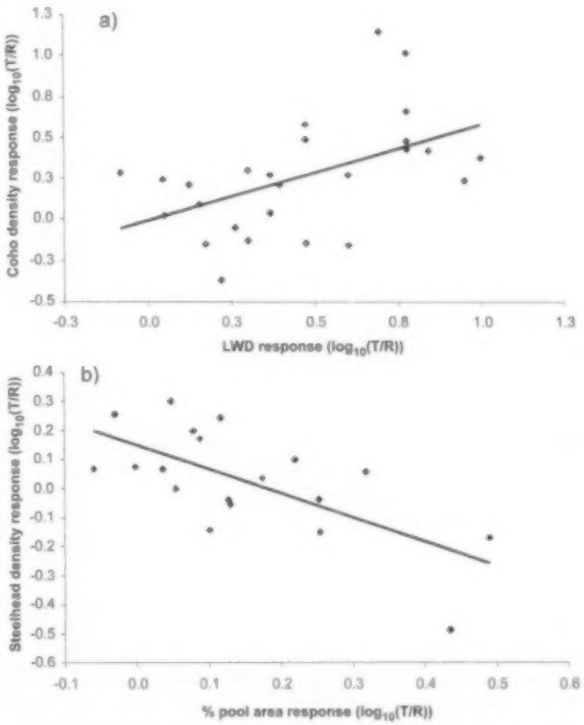
No differences were observed in mean lengths of coho salmon, cutthroat trout, steelhead trout, or trout fry between treatment and reference reaches during summer (paired *t* test, $p = 0.06$, 0.11, 0.56, and 0.50, respectively) or winter ($p = 0.29$, 0.22, 0.37, and 0.16, respectively). However, mean coho length was negatively correlated with coho density ($p < 0.01$, $r^2 = 0.29$) (Fig. 4*b*), but no difference existed between reaches (analysis of covariance, $p = 0.57$). The difference in coho length (treatment minus reference) during summer was negatively correlated with coho density response ($p < 0.01$, $r^2 = 0.29$) (Fig. 4*a*).

Discussion

Physical habitat

Increases in habitat complexity, pools, and slow-water habitats in response to habitat restoration, and specifically LWD placement, have been well documented in western North America (e.g., Riley and Fausch 1995; House 1996; Cederholm et al. 1997). Our results support these findings; an overall increase in pool area, number of pools, and LWD loading was detected in the 30 streams that we sampled. LWD counts differed between summer and winter in some of our sites primarily due to transport of small logs (10 cm in diameter, 1.5–3 m long) in or out of a reach between surveys. However, little difference was detected if only medium and large size-classes (20–50 and >50 cm) were examined.

Fig. 2. Relationship between (*a*) coho salmon response to restoration ($\log_{10}$(treatment (T) density/reference (R) density)) and change in LWD levels ($\log_{10}$(T/R)) for 27 sites inhabited by coho during summer ($y = 0.59x - 0.01$; $p < 0.01$, $r^2 = 0.25$) and (*b*) age 1+ steelhead trout response to restoration ($\log_{10}$(T density/R density)) and change in percent pool area ($\log_{10}$(T/R)) for 20 sites containing 1+ steelhead during summer ($y = -0.83x + 0.15$; $p < 0.01$, $r^2 = 0.45$). Treatment consisted of artificial placement of logs and log structures and reference represents unaltered stream reaches.



Thom (1997) examined physical responses of streams to LWD 1 year after treatment in six of the same streams that we sampled and reported significant increases in number and volume of LWD and number of habitats and deep pools but not in pool area. However, it may take several high-flow events before the channel responds completely to LWD additions (Reeves et al. 1997), and we may have seen a larger physical response than reported by Thom (1997) because we sampled most sites 3–4 years after LWD placement.

Salmonid densities

Most studies on stream restoration or habitat improvement projects have reported significant physical responses to restoration, but evaluation of the responses of juvenile salmonids has been less extensive and the results less consistent. Our sampling of 30 streams (27 utilized by juvenile coho) indicated a 1.8-fold increase in juvenile coho densities during summer in response to LWD placement and a 3.2-fold increase during winter months. Similarly, House et al. (1989) and House (1996) reported increased juvenile coho levels during summer in response to restoration in coastal

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Fig. 3. Relationship between (*a*) juvenile coho salmon response to restoration ($\log_{10}$(treatment (T)/reference (R))) and change in pool area ($\log_{10}$(T/R)) during winter ($y = 0.25x + 0.04$; $p < 0.01$, $r^2 = 0.27$) for 24 sites inhabited by coho during winter and (*b*) trout fry response ($\log_{10}$(T/R)) to restoration and difference in percent pool area ($\log_{10}$(T/R)) for 20 sites inhabited by trout fry during winter ($y = -0.42x + 0.21$; $p = 0.04$, $r^2 = 0.20$). Treatment consisted of artificial placement of logs and log structures and reference represents unaltered stream reaches.

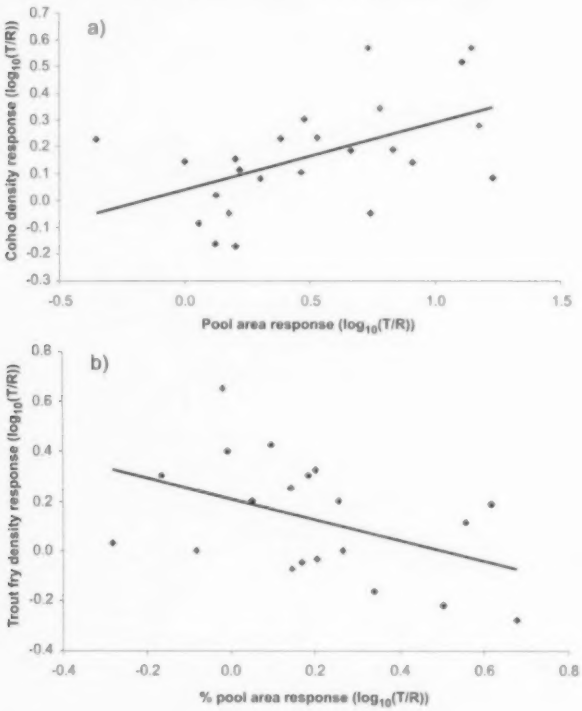
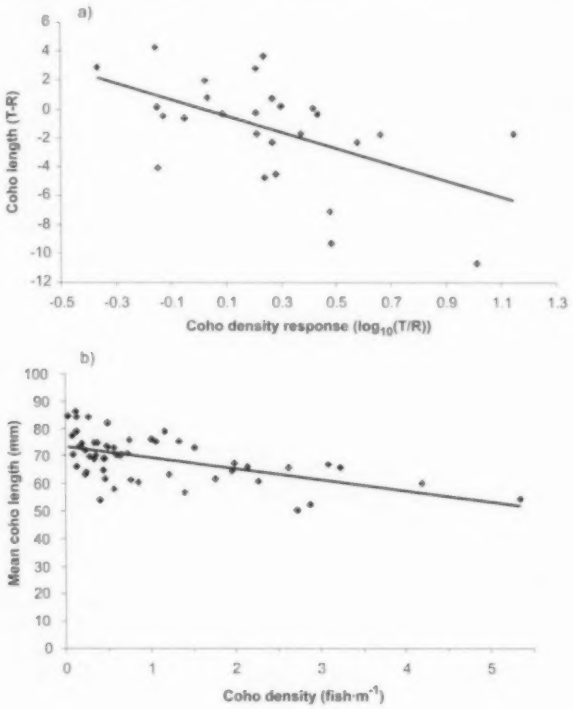


Fig. 4. Relationship between (*a*) mean coho salmon length (treatment (T) – reference (R)) and coho response to restoration ($\log_{10}$(T/R)) for 27 sites containing coho during summer ($y = -5.61x + 0.09$; $p < 0.01$, $r^2 = 0.29$) and (*b*) coho mean length and reach density ($y = 6.10x - 0.07$; $p < 0.01$, $r^2 = 0.29$).



Oregon streams. Nickelson et al. (1992*b*) found similar densities of juvenile coho in constructed and natural pools during both summer and winter in several coastal Oregon streams. However, Cederholm et al. (1997) found no significant difference in juvenile coho densities during summer in response to LWD placement in Porter Creek, Washington, but significantly higher levels of juveniles during winter and smolts during spring. In long-term monitoring of a restoration project in Fish Creek, Oregon, Reeves et al. (1997) found no significant increase in abundance of juvenile coho or coho smolts following restoration. With the exception of Nickelson et al. (1992*b*), these other evaluations occurred on individual streams and may not be broadly applicable elsewhere. Reeves et al. (1997) indicated that many shallow, low-gradient habitats such as glides were eliminated after restoration. We found that glides and shallow pools held the highest densities of juvenile coho during summer. Furthermore, juvenile coho occupy pools, glides, and other low-gradient habitats during summer but are almost exclusively found in pools and slack-water habitats during winter (Hartman 1965; Bustard and Narver 1975*a*; Bisson et al. 1982). Nickelson et al. (1992*a*) indicated that winter habitat was limiting coho salmon smolt production in many Oregon streams, and the larger response that we found for coho salmon during the winter compared to summer supports their findings.

We found that the response of coho to restoration was positively correlated with functioning (pool-forming) LWD during summer and an increase in pool area during winter. It is unclear why functioning LWD was not positively correlated with coho response during winter. However, determining the influence that an individual piece of LWD has on creating a pool is difficult at the higher flows common during our winter surveys. Coho are found almost exclusively in pools and backwater areas during winter, and wood cover is an important element during both summer and winter (Bustard and Narver 1975*a*, 1975*b*; Nickelson et al. 1992*a*). LWD loading is positively correlated with both pool area and pool frequency (Montgomery et al. 1995). Therefore, our results are consistent with the general response of stream channels to LWD and the seasonal habitat preferences of juvenile coho salmon.

We detected an increase in density of juvenile cutthroat trout (age 1+) related to LWD placement in winter and a weak insignificant increase in summer. During summer, cutthroat trout have more general habitat preferences than either steelhead or coho salmon and tend to be found in both pools and low-gradient riffles (Bisson et al. 1982). Therefore, one might expect their response to restoration to be similar to but weaker than that of coho salmon. House et al. (1989) and

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House (1996) reported significant increases in juvenile cutthroat trout densities in response to placement of boulder structures and gabions in coastal Oregon streams during summer. Juvenile coho salmon tend to competitively exclude both steelhead and cutthroat from pools during summer (Hartman 1965; Bisson et al. 1982; Glova 1986), and our results might have differed had we examined more sites inhabited only by cutthroat trout.

Winter densities of cutthroat trout were significantly higher (1.5 times) in treatment reaches. Unfortunately, few studies exist on the winter ecology of cutthroat trout, and we found no studies on the response of coastal cutthroat trout to restoration in winter months. Bustard and Narver (1975*b*) found that during winter, juvenile cutthroat occupied pools with cover. Glova (1986) found that agonistic behavior between cutthroat and coho was high during summer and led to habitat segregation, but aggression was low during winter and both species utilized pools, consistent with the observations of Bustard and Narver (1975*b*). The seasonal differences that we observed in the response of cutthroat trout to LWD placement are most likely due to a shift in habitat use and competition from summer to winter. We observed coho, steelhead, and cutthroat almost exclusively in pools during winter, so factors affecting pools might be expected to influence winter densities or distributions.

Steelhead trout densities did not differ between treatment and reference reaches during summer. Case studies examining steelhead response to LWD and boulder placement in individual streams have shown varying results. Cederholm et al. (1997) found no change in juvenile steelhead densities during summer or winter following LWD placement. Similarly, House (1996), Moreau (1984), and Chapman (1996) found no significant difference in steelhead parr or fry densities during summer following placement of in-stream structures. However, steelhead response to LWD placement in our study was negatively correlated with changes (increase) in pool area and positively correlated with changes (decrease) in riffle area. This is consistent with studies on habitat use that indicate that steelhead occupy riffles and fast-water habitats during summer (Bisson et al. 1982). Thus, the physical responses to LWD placement that tend to benefit coho salmon (increased pool area and decreased riffle area) may decrease juvenile steelhead densities at those sites in summer.

In contrast with summer, winter steelhead densities were significantly higher (1.7 times) in treatment than in reference reaches. Only Cederholm et al. (1997) and Reeves et al. (1997) evaluated winter or spring response of juvenile steelhead to LWD additions, but neither found significant increases in juveniles or smolts. During winter, we observed juvenile steelhead, cutthroat, and coho primarily in pool habitats whereas in summer, steelhead were generally found in low-gradient riffles and glides. However, no significant relationship existed between change in pool area (treatment minus reference) and steelhead densities (treatment minus reference), even though our observations and data from other studies indicate that steelhead show a strong preference for pools and woody cover during winter (Hartman 1965; Bustard and Narver 1975*a*). The lack of a significant relationship between steelhead response to restoration and specific physical variables during winter may be due to the large natural variability among sites or our inability to quantify microhabitats (substrate, velocity preferences, etc.) to which steelhead were responding. However, our results indicated that during winter, age-1+ steelhead benefit from LWD placement, and data on habitat preferences suggest that it is most likely due to an increasing preference for pools and LWD cover during winter.

We found no significant increase in trout fry (age-0 cutthroat and steelhead) densities during either summer or winter in relation to restoration. House et al. (1989) reported increases in trout fry densities following placement of in-stream structures, but Hamilton (1989), House (1996), and Cederholm et al. (1997) reported no increase in trout fry following restoration, and Reeves et al. (1997) found a significant decline in steelhead fry following restoration. Bisson et al. (1988) indicated that age-0 steelhead trout showed no strong preference or avoidance of most habitat types during summer, except backwater pools where they were most abundant. Similarly, they found that age-0 cutthroat tended to avoid riffles and prefer pools and glides. Age-0 steelhead or rainbow trout tend to occupy stream margins during summer and winter (Hartman 1965). Moore and Gregory (1988) found that cutthroat trout fry densities were positively correlated with lateral (edge) habitat and increased 2.2-fold when lateral habitat was experimentally increased. We did not quantify edge or lateral habitats, although our general observation was that little change occurred in lateral habitats as a result of LWD placement and the creation of pools by LWD may have eliminated some shallow water edge habitats. Trout fry response to LWD placement in our study was negatively correlated with pool area during winter, suggesting that increasing deepwater habitats may eliminate some fry rearing areas.

Fish length

Mean fish length did not differ between treatment and reference reaches. However, coho were generally smaller in treated stream reaches whereas age-1+ cutthroat and steelhead tended to be slightly larger. The difference in fish length between treatment and reference reaches was positively correlated with density for coho salmon, indicating that LWD placement led to more but smaller juvenile coho during summer months. Coho salmon growth in streams is inversely related to density (Fraser 1969), and overwinter survival and smolt to adult survival within a population are also size dependent (Quinn and Peterson 1996). To the extent that restoration leads to density-dependent reduction in growth, there may be smaller responses in adult abundance than might be projected from the increased juvenile densities.

Other factors influencing fish response

The purpose of most stream restoration efforts is to increase the abundance of fish. However, evaluations of restoration (including ours) tend to quantify fish response at small spatial scales. If the improved habitat simply concentrates fish that are moving among reaches and reduces their growth, the consequences for the population may be negligible. Consistent with this concern, Kahler (1999) reported that about half the juvenile coho salmon moved at least one habitat unit during the summer in three western Washington streams. Moreover, fish in poor-quality habitat (smaller, shallower pools) were more likely to move than those in

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larger pools. A mark–recapture study conducted in one of our study streams found little or no movement of fish between the treatment and reference reaches during summer and winter (P. Roni, unpublished data). Riley and Fausch (1995) reported increases in adult trout numbers following restoration in six Wyoming streams, but recovery of marked and unmarked fish suggested that movement rather than survival was responsible for much of the increase. However, Riley and Fausch (1995) acknowledged that they did not monitor the sites long enough to determine whether there was a long-term increase in fish production due to LWD placement. Therefore, while movements may explain some differences in fish densities between treatment and reference reaches, there may also have been changes in survival, but we have no direct way to distinguish these factors.

Hamilton (1989) reported a twofold increase in steelhead parr in treated stream reaches, while numbers in untreated reaches decreased by half over a 2-year period. Initially, placement of in-stream structures or LWD might lead to redistribution of juvenile and resident fish, and it may take several years for fish populations to fully respond. Reeves et al. (1991*b*) suggested that monitoring for two generations might be needed to detect population response for anadromous salmonids in an individual stream. We sampled most sites 3–7 years (mean = 4.4) after treatment so that the full biological response was likely to have occurred.

It is possible that differences in LWD placement technique or initial LWD abundance may account for some of the differences that we observed. Most of the sites taht we sampled in Washington consisted of "engineered LWD structures" such as log weirs and deflectors that were held in place with rebar or cable whereas most of the Oregon sites were artificially created LWD jams with minimal use of cable. Sites in Oregon were generally selected for LWD placement only if stream surveys had indicated that there was little to no LWD present (Thom 1997). In contrast, some Washington sites had moderate levels of natural LWD in reference and treatment reaches. These sites were selected for treatment due to reasons other than just LWD levels such as ease of access, available funds, etc. Differences in fish response between states (regions) were most pronounced for coho salmon in winter; abundance in treatment reaches was 4.3 times that in reference reaches in Oregon sites compared with 2.3 in Washington sites. While we were not able to definitively attribute differences in fish response to region or structure type, sites that had low levels of LWD prior to treatment generally had the largest physical and biological responses. This is supported by the positive relationship between coho response and difference in LWD numbers between treatment and reference sites.

The placement of LWD has been shown to trap gravels (House et al. 1989), and the placement of LWD likely led to differences in substrate size and possibly number of spawning salmonids in treatment and reference reaches. We did not quantify spawning salmonids or their habitat, but spawner abundance of coho was reportedly low in some Oregon streams that we sampled. Low numbers of adults may result in compensatory high survival rates of fry, and large-scale movements of juvenile coho during spring and fall (Kahler 1999) would lead to redistribution among treatment and reference reaches. The juvenile densities that we observed suggest that these streams were at moderate seeding levels, and we feel that differences between reaches are more likely related to differences in physical habitat rather than differences in spawner abundance.

Reeves et al. (1991*b*) suggested that response to restoration should be monitored at a watershed level through smolt trapping rather than at a reach scale. However, most in-stream projects occur at the reach scale (100–500 m), and monitoring at a watershed level is unlikely to detect changes in fish abundance or habitat use at the reach level. Smolt trapping treatment and reference reaches, such as that by Cederholm et al. (1997), would have provided estimates of smolt production but would have been prohibitively expensive because of the large number of streams that we sampled. Because most coho are likely to have smolted shortly after our winter surveys, they provide reasonable estimates of smolt production from the study reaches. Ultimately, a study combining both reach-scale and watershed-scale juvenile and smolt production estimates is needed to estimate response at different scales and provide additional information on fish movements within restored and unrestored sections of a watershed.

In summary, our results provide strong evidence that artificially placed LWD leads to significantly higher densities of juvenile coho in summer and winter and higher densities of cutthroat and steelhead during winter, especially at sites deficient in wood to begin with. However, density-dependent growth of coho salmon may reduce the net benefit to the population if subsequent survival is size related. Summer densities of steelhead may be reduced by artificially placed LWD, but this may be more than compensated for by increases in winter rearing areas and densities. While the study design that we employed was not designed to determine the effectiveness of individual projects, it provides insight into the factors that make projects successful. The relationship between coho abundance and pool area and functioning wood indicates that projects that dramatically increase pool area or LWD creating pools will provide the largest increases in fish abundance. However, we focused on forested sites, and the response in highly urbanized and agricultural areas may be different. Finally, our results in no way negate the need to focus on restoring natural processes that create and maintain salmon habitat rather than relying on in-stream manipulations that can be costly and not address the ultimate factors limiting habitat quality and salmonid production.

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Copper metabolism and gut morphology in rainbow trout (*Oncorhynchus mykiss*) during chronic sublethal dietary copper exposure

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Abstract: Juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed to 11 (control), 300 (medium), and 1000 μg Cu·g^{-1} (high) (as $CuSO_4 \cdot 5H_2O$) in the diet for 28 days at a daily ration of 4% wet body weight, with a background waterborne Cu concentration of 3 μg·L^{-1}. There was no effect of dietary Cu on growth, condition factor, or food conversion efficiency. Whole-body Cu content increased continuously over the exposure period in all groups and was twofold and fourfold higher than controls at day 28 for the medium- and high-Cu diets, respectively. Copper accumulated mainly in liver and gut tissue, with the latter stabilizing by day 14. Accumulation also occurred in gill, kidney, and carcass. Plasma Cu concentration was not different from the controls whereas Cu in bile was greatly elevated, an indication of increased hepatobiliary excretion. Dietary Cu pre-exposure decreased the uptake of waterborne Cu across the gills, providing the first evidence of homeostatic interaction between the two routes of uptake. Electron microscopic observations of the midintestine revealed numerous mitochondria, lysosomes, lamellated bodies, and extensive lamellar processes in the enterocytes. Apoptosis, mitosis, and eosinophilic granule cells were more apparent in Cu-exposed fish.

Résumé : Des jeunes Truites arc-en-ciel (*Onchorhynchus mykiss*) ont été exposées pendant 28 jours à des concentrations de 11 (témoin), de 300 (moyenne) ou de 1000 μg Cu·g^{-1} (élevée), sous forme de $CuSO_4 \cdot 5H_2O$, dans des rations alimentaires équivalant à 4% de leur masse corporelle humide et dans une eau contenant des concentrations d'arrière plan de Cu de 3 μg·L^{-1}. Le Cu alimentaire est resté sans effet sur la croissance, le coefficient d'embonpoint et l'efficacité de la conversion de la nourriture. Le contenu corporel total en Cu a augmenté de façon constante chez tous les groupes au cours de l'exposition et, au jour 28, il était deux fois plus élevé dans le groupe à exposition moyenne que chez le groupe témoin et de quatre fois plus élevé chez le groupe à exposition élevée. Le Cu s'accumulait principalement dans le foie et dans les tissus du tube digestif, atteignant un palier chez ces derniers au jour 14. Il y avait aussi une accumulation dans les branchies, les reins et la carcasse. Les concentrations plasmatiques de Cu n'ont subi aucune hausse; en revanche, les concentrations dans la bile ont crû fortement, ce qui indique une augmentation de l'excrétion hépatobiliaire. Une exposition préalable au Cu alimentaire diminuait l'incorporation du Cu de l'eau par les branchies, ce qui constitue une première mention d'une interaction homéostatique entre ces deux voies d'incorporation. L'examen du tube digestif moyen au microscope électronique a révélé la présence dans les entérocytes de nombreux lysosomes, mitochondries, corpuscules lamellés et grands processus lamellaires. L'apoptose, la mitose et la présence de granulocytes éosinophiles étaient fréquentes chez les poissons exposés au Cu.

[Traduit par la Rédaction]

Introduction

Fish assimilate Cu from both water and diet (Dallinger et al. 1987). While the uptake and effects of waterborne Cu are fairly well understood (McDonald and Wood 1993), effects of dietary Cu in fish have been less thoroughly investigated (Handy 1996). A few studies have highlighted a micronutrient requirement for Cu (Ogino and Yang 1980; Murai et al. 1981), while others have recognized dietary exposure as a source of Cu intoxication in fish (Woodward et al. 1994, 1995; Handy 1996). Available dietary Cu exposure data indicate that bioavailability is very low (3% in rainbow trout (*Oncorhynchus mykiss*)) and toxic effects occur at much higher exposure levels relative to waterborne exposures (Lanno et al. 1985; Julshamn et al. 1988; Handy 1992). It has been argued that the gastrointestinal tract offers a strong barrier to dietary toxicants and effectively regulates metal uptake (Berntssen et al. 1999). Although the majority of the studies on dietary Cu exposure in fish have assessed growth and tissue-specific accumulation, results have been rather variable (Handy 1992; Mount et al. 1994; Berntssen et al. 1999) and probably reflect the differences in experimental designs, diet variables such as Cu concentrations and bioavailability, and the length of the exposure.

Interactions between dietary and waterborne Cu uptake are likely important in Cu homeostasis in fish. Unfortunately, nothing is known about the effects of dietary Cu preexposure on subsequent waterborne Cu uptake or vice versa. Previous studies that attempted to delineate effects of waterborne and dietary Cu exposure (Miller et al. 1993; Farag et al. 1994) focused mainly on tissue metal accumula-

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tion attending Cu exposure through the two routes. The problem with such studies is distinguishing Cu of dietary uptake from that of waterborne uptake. Application of direct measurements of Cu uptake using radioisotope methodologies (Wood 1992) not only permits this but also distinguishes Cu of recent uptake from Cu accumulated over the long term.

Exposure to elevated dietary metal levels is often accompanied by morphological changes in the gut, but there are only a few studies on Cu. Effects of a dietary metal mixture (including Cu) on rainbow trout gut morphology, as described by Woodward et al. (1995), include gut impaction, swelling, ulceration, and epithelial lifting. Recently, Berntssen et al. (1999) reported increased apoptosis and cell proliferation in the ileum of Atlantic salmon (*Salmo salar*) parr exposed to elevated dietary Cu. Additional morphological studies are desirable to more fully describe the impact of dietary Cu on the fish gut.

In the present study, we evaluated tissue-specific partitioning and bioaccumulation of Cu following dietary exposure at environmentally realistic sublethal levels. Secondly, we assessed the impact of dietary Cu exposure on subsequent uptake and distribution of waterborne Cu using a sensitive radioisotope methodology that distinguishes recently accumulated Cu from that already present in tissues prior to exposure. Finally, we described the morphological changes occurring in the midintestine during chronic elevated dietary Cu exposure.

Materials and methods

Experimental animals

One hundred and twenty juvenile rainbow trout (weight and length (mean ± SEM) = 37.24 ± 1.20 g and 14.86 ± 0.20 cm, respectively) were transferred from laboratory stock (originally from Humber Springs Trout Farm, Ontario) and equally divided into three 80-L experimental tanks supplied with a throughflow of aerated dechlorinated Hamilton city tap water (Na^+, 13.8 mg·L^{-1}; Cl^-, 24.8 mg·L^{-1}; Ca^{2+}, 40 mg·L^{-1}; HCO_3^-, 115.9 mg·L^{-1}; pH, 7.9–8.2; background Cu, 3 μg·L^{-1}) at flow rates of 1.2 L·min^{-1}. Seven days before starting the experiment, all the fish were anesthetized with 1% tricaine methanesulphonate (MS 222) and individually marked by introducing alcian blue dye (0.06 g·mL^{-1} in nanopure water) spots on their undersides with Panjet (Wright Dental Manufacturing Co., Dundee). This facilitated fish identification and determination of specific growth rates.

Diet preparation

Copper-enriched diets were "made" in-house by grinding commercial trout chow (5 point Regular Trout Grower Pellets; composition: 40% crude carbohydrate, 42% crude protein, 1% calcium, 0.75% phosphorus, and 0.35% sodium (Martin's Feed Mill Ltd., Elmira, Ont.)) and mixing it with Cu (as $CuSO_4 \cdot 5H_2O$; Fisher Scientific) to give nominal diet concentrations of 300 (medium) and 1000 μg Cu·g^{-1} (high). The food was then extruded through a pasta maker, air-dried, and broken into small pellets (approximately 8 mm^3) by hand. The actual Cu content of the diets (mean ± SEM) as determined by atomic absorption spectroscopy was 277.8 ± 5.9 (n = 5) and 1041.9 ± 17.5 μg·g^{-1} (n = 5) for the medium- and high-Cu diets, respectively. These dietary levels of exposure were chosen to fall within environmentally realistic levels (Dallinger and Kautzky 1985) and above maximum tolerable levels (Lanno et al. 1985), respectively. Control diet consisted of the same commercial fish chow treated in the same way but not supplemented with Cu. Cu concentration of the control diet was 11.4 ± 0.2 μg·g^{-1} (n = 5).

Feeding, presampling procedure, and sampling regime

Fish were fed twice daily in the morning (08:00–09:00) and evening (18:00–19:00) at 2% wet body weight, totaling 4%·day^{-1}, and water temperature was maintained at 14 ± 1°C throughout the experimental period. Visual examination during feeding revealed that all fish readily ingested the diets; there was no avoidance of the high-Cu diet. Fecal material was siphoned within 1 h of feeding, and the flow-through system continuously flushed away any fecal matter deposited between the feeding times. Copper concentrations in the exposure tanks before and after feeding ranged from 2.89 to 3.21 μg·L^{-1} in all groups; there were no differences before and after feeding. Sampling was done at the start of the exposure (day 0) and at days 14 and 28. Prior to sampling, all the fish were anesthetized with 1% MS 222, identified, and their weights and lengths taken individually. After recovery from the anesthesia, the fish were returned into their respective experimental tanks and starved for 24 h to clear the gut of ingesta. During the starvation period, fecal material was siphoned from the tanks twice every 12 h to minimize any chance of coprophagy.

Waterborne ^{64}Cu exposure

Measurement of waterborne Cu uptake, by means of ^{64}Cu flux determination, preceded terminal sampling at all sampling times. The radioisotope ^{64}Cu (as $Cu(NO_3)_2$, $t_{1/2}$ = 12. 65 h) was prepared at the McMaster Nuclear Reactor, Hamilton, Ont. Fish (n = 10) from the control group and the two dietary Cu exposed groups were moved into separate tanks containing 20 L of static aerated dechlorinated Hamilton city tap water and allowed to settle for 1 h. Subsequently, 0.7 μCi ^{64}Cu·L^{-1} (specific activity 0.35 μCi·g^{-1}) (1 μCi = 37 kBq) was introduced into each tank and the exposure carried out for 12 h. A time course study demonstrated that 12 h of exposure was adequate to allow measurable uptake of the Cu into the target organs and whole body. Furthermore, whole-body uptake was linear over the 12-h period, demonstrating that a 1-h settling period prior to exposure was adequate and that there was no deterioration of conditions that affected Cu uptake over the 12 h. The radioisotope dosage administered added a total concentration of 0.2 μg Cu·L^{-1} in the water and was chosen to ensure that the total water Cu was not substantially elevated above the ambient level of 3 μg·L^{-1}.

During the 12-h static ^{64}Cu flux, excretory Cu significantly elevated the water Cu levels above the ambient concentration. To overcome this problem, a separate ^{64}Cu flux experiment was performed on fish that had been exposed to either control or high-Cu diet for 14 days. In this experiment, 50 individually marked fish (25 control and 25 high dietary Cu exposed) were exposed to ^{64}Cu for 12 h in the same flux chambers so that total Cu concentrations in the water would be identical for the two groups. Five different water Cu concentrations (1.3, 2.5, 5.0, 6.2, and 12.0 μg·L^{-1}) were tested with five fish from each treatment in each flux chamber.

When it became evident that waterborne Cu uptake was strongly influenced by body size, an additional experiment was performed with 144 juvenile rainbow trout ranging in body mass from 0.5 to 77 g. ^{64}Cu flux was measured under control conditions (control diet, water Cu approximately 3 μg·L^{-1}) using methods identical to those outlined above.

Sampling

Fifteen minutes after introduction of ^{64}Cu to the flux tanks, a 10-mL water sample was taken from each tank. After 12 h of exposure to ^{64}Cu, a second 10-mL water sample from each tank was taken. Subsequently, the fish were anesthetized with 1% MS 222 and a blood sample obtained by caudal puncture into heparinized 1-mL syringes fitted with 23-gauge needles. An aliquot of 50 μL of

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each blood sample was immediately centrifuged for 4 min at 13 000 × *g* to separate plasma. The fish were then killed by a blow to the head and the entire gill baskets, liver, gut, kidney, muscle, and the rest of the carcass dissected out and collected into separate preweighed scintillation vials or Eppendorf tubes. Before dissecting out the liver, bile was collected by aspiration from the gallbladder into a 1-mL syringe fitted with a 23-gauge needle. In addition, the carcass and gills were rinsed in nanopure water to remove surface-bound radioactivity.

Analysis

^{64}Cu activity in the tissue and water samples was measured on a Canberra-Packard Minaxi gamma counter with an onboard program for correcting for decay. Subsequently, the tissues were digested overnight at 70°C with six volumes of 1 N nitric acid (Fisher Scientific, trace metal grade) and then centrifuged for 4 min at 13 000 × *g*. A subsample of the supernatant was diluted suitably with 0.5% nitric acid. Total tissue Cu concentration was determined by atomic absorption spectrophotometry (Varian AA-1275 with GTA furnace atomizer) using a 10-µL injection volume and the operating conditions specified for Cu by the manufacturer. Certified Cu standards (National Research Council of Canada) run at the same time were within the specified range. Under the conditions of the analyses (taking dilution factors into account), the detection limits for water and tissue were 0.3 and 0.6 $\mu g \cdot g^{-1}$, respectively, far below experimental values measured in this study.

Calculations

Whole-body total Cu concentration was calculated by dividing the sum of Cu contents (concentration multiplied by weight or volume) of all the tissues plus the carcass by the sum of weights of all the tissues plus the carcass. Fish Cu content was calculated by multiplying whole-body (fish) Cu concentration by the fish weight.

The ^{64}Cu uptake of the tissues was calculated on individual fish basis using the equation

$$(1) \qquad a(bc^{-1})^{-1}$$

where a is the ^{64}Cu counts per minute per gram of tissue, b is the ^{64}Cu counts per minute per litre of water, and c is the total Cu concentration in water in micrograms per litre. Proportional distribution was calculated from the product of eq. 1 for each tissue and that tissue weight divided by the sum of these values for all tissues plus carcass.

"Previous compartment analysis," as outlined by Grosell et al. (1997), was used to trace the movement of Cu between different body compartments. Newly accumulated Cu in each tissue or organ was calculated on an individual fish basis using the equation

$$(2) \qquad a(de^{-1})^{-1}$$

where a is ^{64}Cu counts per minute per gram of tissue, d is ^{64}Cu counts in the previous compartment (counts per minute per litre), and e is the total Cu concentration in the previous compartment (micrograms per gram or micrograms per litre, respectively). Previous compartments, based on the criteria of Grosell et al. (1997), were water for gill, gill for plasma, plasma for liver, gut tissue, kidney, muscle, and carcass, and liver for bile.

Specific growth rate (SGR) was calculated for each 2-week period (0–14 and 15–28 days) using the formula

$$(3) \qquad \mathrm{SGR} = 100(\ln(\mathrm{wt}_2) - \ln(\mathrm{wt}_1))t^{-1}$$

where wt_1 and wt_2 are individual fish weights at the start and end of the each growth period, respectively, and t is the time interval in days. Fish were identified by their distinctive marks and weighed individually.

Condition factor (k) for individual fish was calculated using the formula

$$(4) \qquad k = 100(\mathrm{weight} \times \mathrm{length}^{-3}).$$

Food conversion efficiency (FCE) on a per tank basis was calculated as

$$(5) \qquad \mathrm{FCE}\ (\%) = 100(\mathrm{weight\ gain} \times \mathrm{food\ eaten}^{-1}).$$

Transmission electron microscopy

At each sampling time (0, 14, and 28 days), three additional fish per treatment (27 in total) were killed with an overdose of MS 222 and the gastrointestinal tract exposed. Control and experimental fish were processed simultaneously. The midintestine was identified as the section of the intestine between the last pyloric cecum and the start of the distal intestine (darker region with larger diameter and annular rings), dissected out, immediately flushed with Cortland saline (Wolf 1963), and fixed by immersion for 2 h in 5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, osmolarity, 230 mosmol. Subsequently, it was cut into four equal regions, which were further subdivided into four medium-sized blocks. Each block was then diced into small pieces, approximately 1 mm^3 in size. These pieces were then fixed further overnight in greater than 10 times volume of the same solution. Ten such pieces per fish were selected randomly and postfixed in 1% buffered osmium tetraoxide for 4 h at 4°C. The tissues were subsequently dehydrated in a series of ethanol solutions from 50 through 100% and embedded in Spurr's resin. Five-micrometre-thick semithin monitor sections were obtained and stained with toluidine blue for light microscopic observation. Thin sections (80 nm thick) were then obtained and mounted on Cu grids, stained with uranyl acetate and lead citrate, and viewed under a JEM–1200EXII transmission electron microscope at 80 kV.

Statistical analysis

All data except the qualitative microscopic observations and food conversion efficiency are presented as means ± SEM. Effects of different oral exposure of Cu on growth, tissue Cu concentration, and subsequent waterborne Cu uptake at each sampling point were assessed using a two-way analysis of variance with time and diet Cu concentration as variables. Percentage data were subjected to arcsine transformation prior to statistical testing. Significance was set at $p < 0.05$. The Student–Newman–Keuls pairwise multiple comparison procedure was used to make comparisons between measurements.

Results

Growth and mortality

All the fish maintained high rates of growth during the exposure. Mean weights rose from about 37 g at the start of the exposure to about 70 g, while length increased from 15 to 18 cm (Table 1). Under our conditions of exposure, dietary Cu concentration as high as 1042 $\mu g \cdot g^{-1}$ had no significant effect on specific growth rate, condition factor, and food conversion efficiency. However, nonsignificant growth stimulation and inhibition were observed with medium- and high-Cu diets, respectively. A similar trend was evident for condition factor and food conversion efficiency (Table 1). No mortality occurred during the exposure.

Copper bioaccumulation and partitioning

When expressed on an individual fish basis, Cu content increased continuously with normal growth throughout the experiment in the control fish as well as in the Cu-exposed fish (Fig. 1). However, by day 28, total Cu content per fish was about twofold higher in the medium-Cu diet group and

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Table 1. Means ± SEM (n) starting and final wet weights and lengths, specific growth rate (SGR), condition factor (k), and food conversion efficiency (FCE) for control and Cu-exposed rainbow trout.

	Diet Cu content		
Biological index	Control (11 μg·g^{-1})	Medium (300 μg·g^{-1})	High (1000 μg·g^{-1})
Starting weight (g)	36.99±1.93 (40)	37.68±2.16 (41)	37.04±2.219 (40)
Final weight (g)	63.32±5.73 (17)*	75.99±4.98 (18)*	65.69±5.15 (17)*
Starting length (cm)	14.67±0.40 (41)	14.94±0.33 (41)	14.99±0.33 (40)
Final length (cm)	17.57±0.49 (17)*	18.72±0.34 (18)*	18.53±0.84 (17)*
SGR (%·day^{-1})			
Days 0–14	2.44±0.12 (25)	2.69±0.14 (27)	2.16±0.14 (27)
Days 15–28	2.41±0.12 (14)	2.50±0.23 (13)	2.40±0.16 (13)
k			
Day 0	1.14±0.06 (40)	1.11±0.04 (41)	1.07±0.03 (40)
Day 14	1.10±0.02 (30)	1.15±0.02 (31)	1.09±0.01 (30)
Day 28	1.11±0.01 (17)	1.13±0.02 (18)	1.06±0.06 (17)
FCE (%)			
Days 0–14	72.8 (25)	81.1 (27)	64.2 (27)
Days 15–28	69.0 (14)	69.9 (13)	66.7 (13)

*Significant difference ($p < 0.05$) from starting values.

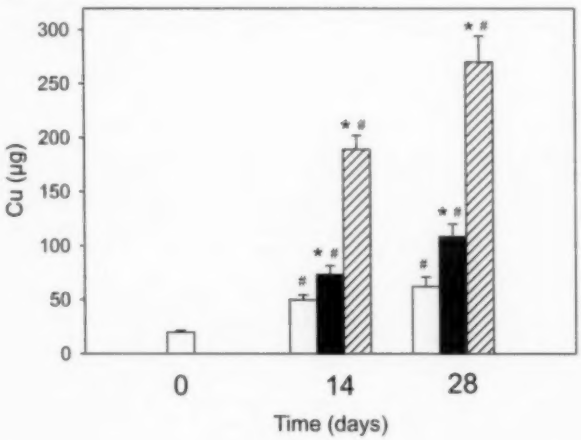
fourfold higher in the high-Cu diet group relative to the simultaneous controls. Since growth rates were unaffected by these treatments, whole-body Cu concentrations exhibited similar trends (Table 2). However, Cu concentrations in the gut tissue rose to a much greater extent, from about 1 μg·g^{-1} in controls to approximately 5 μg·g^{-1} for the medium-Cu diet and 25 μg·g^{-1} for the high-Cu diet on days 14 and 28. Uptake and transport of Cu to internal organs occurred with significant accumulation in the liver, bile, kidney, gills, muscle, and carcass. Only in the gut tissue and bile did Cu accumulation appear to reach steady state. Despite this pattern of widespread accumulation, plasma Cu remained constant between 0.5 and 0.7 μg·mL^{-1} throughout the exposure.

The proportional distribution of total Cu in the organs and tissues sampled is shown in Table 3 based on measurements of the absolute amounts of Cu in the different organs and in the whole animal. The key organs and tissues for Cu accumulation were the liver, gut, and carcass (which includes muscle). In control animals, the liver and carcass together contained 75–85% of the Cu, while all the other organs together contained 15–25% of the Cu. Following dietary Cu exposure, the contribution of the carcass decreased and Cu was distributed mainly in the liver and gut tissue. The contribution of these two tissues to total body Cu was 65–75% in fish exposed to the medium-Cu diet and 85% in fish exposed to the high-Cu diet. Gills contributed less than 3.5% of the total Cu in control fish, and fish exposed to dietary Cu had a significantly lower proportion of the Cu in gills. The kidney accounted for less than 2% of the total Cu in all the groups.

Waterborne ^{64}Cu uptake

Exposure to waterborne ^{64}Cu for 12 h resulted in detectable levels of radioactivity in all the body tissues sampled (Fig. 2A). In the early stages of the exposure, gills showed high levels of radioactivity, but with time, greater radioactivity was registered in internal organs, especially the liver. By the end of the 12 h, the ^{64}Cu concentrations were highest in the liver followed in decreasing order by the gill, gut, and carcass. Uptake into the whole body (sum of activities in all the tissues over total weight of all the tissues) increased linearly with time over the 12-h period (Fig. 2B).

Fig. 1. Total Cu content per fish in control and dietary Cu exposed rainbow trout at days 0, 14, and 28. Open bars, control; solid bars, 300 μg Cu·g diet^{-1}; hatched bars, 1000 μg Cu·g diet^{-1}. Values are means ± SEM, n = 10 per treatment per time. An asterisk indicates a significant difference from the corresponding control; a pound sign indicates a significant difference from the day 0 control.



Two complications arose in the measurement of ^{64}Cu uptake from the water. Firstly, weight-specific uptake clearly declined with body mass. Secondly, levels of Cu in the water varied among different trials due to differential Cu excretion by the fish, as outlined below. Therefore, two additional experiments were performed. In the first, analysis of Cu uptake measurements taken from 144 unexposed fish of different sizes ranging from 0.5 to 77 g showed a clear nonlinear dependence of mass-specific uptake (y, nanograms per gram) on body mass (x, grams) that was best described by a negative exponential relationship (Fig. 3A): $y = 0.179 \exp(-0.0743x)$ ($r^2 = 0.45$, $p < 0.0001$). This relationship was used to correct

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Table 2. Total Cu concentration (means ± SEM, μg·g wet weight^{-1} or μg·mL^{-1}, n = 10 per treatment) in tissues and whole body of control and dietary Cu exposed rainbow trout after 0, 14, and 28 days of exposure.

	Gill	Liver	Gut	Kidney	Muscle	Carcass	Bile	Plasma	Whole body
Day 0, control	0.69±0.04	16.85±1.89	1.26±0.13	1.57±0.09	0.36±0.02	0.48±0.05	13.94±1.11	0.70±0.07	0.63±0.06
Day 14									
Control	0.54±0.02	18.57±2.12	2.29±0.23	2.77±0.25	0.31±0.02	0.34±0.04	7.04±0.76	0.56±0.03	0.93±0.06
Medium	0.65±0.06*	29.91±3.71*	3.20±0.62	4.48±1.09	0.27±0.01	0.51±0.07*	16.36±1.7*	0.52±0.03	1.84±0.32*
High	0.94±0.16*	62.67±12.28*	25.96±2.42*	5.31±1.12*	0.37±0.04	0.48±0.03*	28.54±3.01*	0.52±0.02	3.74±0.16*
Day 28									
Control	0.91±0.07	38.40±6.43	2.19±0.73	1.70±0.32	0.26±0.02	0.36±0.04	10.75±0.99	0.50±0.04	1.46±0.24
Medium	1.22±0.04*	45.18±6.87	5.41±1.03*	1.73±0.16	0.32±0.04	0.45±0.03	15.39±1.7*	0.52±0.01	1.72±0.15
High	1.37±0.09*	100.27±22.51*	23.58±2.44*	9.98±3.3*	0.32±0.01*	0.70±0.12*	27.73±2.89*	0.50±0.01	5.24±0.53*

*Significant difference ($p < 0.05$) relative to the respective control.

the data for all fish in the second experiment to a common weight of 10 g.

In this experiment, control and high dietary Cu exposed fish were exposed to ^{64}Cu in the same flux chambers at a range of identical water Cu concentrations. The results (Fig. 3B) demonstrate that dietary Cu pre-exposure significantly reduced subsequent branchial ^{64}Cu uptake. In addition, Cu uptake increased linearly with water Cu concentration over the range of water Cu tested in both control and experimental groups.

During the initial 12-h flux experiments where the control and Cu-treated fish were kept in separate chambers, net excretion of Cu from the medium and high dietary Cu exposed groups significantly elevated the water Cu concentration above the ambient level of 3 μg·L^{-1}. Furthermore, in different trials, the extents of these elevations were different. Thus, absolute rates of Cu uptake from the water could not be validly compared between treatments, and the ^{64}Cu data could only be used to compare relative internal distribution of ^{64}Cu with that of total Cu. Treatment-related differences for ^{64}Cu distribution at day 28 were relatively minor, but there were major differences in comparison with total Cu distribution (Fig. 4). In particular, the gills contained approximately 20% of ^{64}Cu but only 2% of the total Cu. Furthermore, the carcass contained about 60% of the ^{64}Cu but only 20% of the total Cu. The other major differences were in gut and liver, which each contained about 5–10% of the ^{64}Cu whereas about 50% of total Cu was in the liver and 25% in the gut. Similar patterns were seen at day 14 (data not shown).

Newly accumulated Cu

Newly accumulated Cu in each of the tissues sampled calculated using the previous compartment analysis of Grosell et al. (1997) is shown in Table 4. In both the medium and high dietary Cu exposed groups, all the tissues analyzed exhibited newly accumulated Cu concentrations that were similar to those of the respective controls, except for the liver of the high dietary Cu group at day 28 and the gut of the medium dietary Cu group at day 14. In all cases, the level of newly accumulated Cu was one to two orders of magnitude lower than the corresponding total Cu concentration (note the different units in Tables 2 and 4) except for bile and plasma. For bile and plasma, the calculated values of newly accumulated Cu were in excess of the corresponding total Cu concentrations, indicating the dynamic turnover in these two compartments.

Midintestinal morphology

Representative electron micrographs of midintestine from control (n = 15) and Cu-exposed (n = 12) rainbow trout are shown in Fig. 5. Normal midintestinal epithelium comprised absorptive cells, the enterocytes, and mucous cells (Figs. 5A and 5B). Enterocytes possessed numerous closely packed microvilli (brush border) and were joined by typical epithelial cell junctional complexes, while the mucous cells were densely packed with secretory granules of variable size and electron density.

No differences were noted in midintestinal structure between the medium and high dietary Cu exposed groups or between day 14 and day 28. The most prominent effect of

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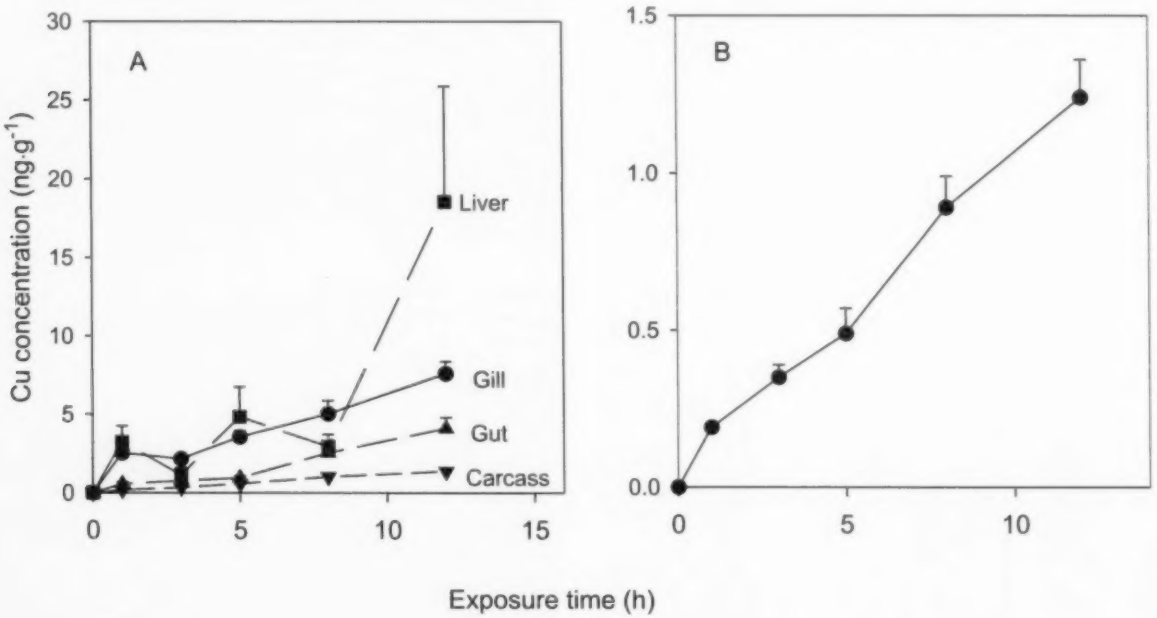
Table 3. Proportional distribution (%, means ± SEM) of total body Cu in control and dietary Cu exposed rainbow trout.

	Gill	Liver	Gut	Kidney	Carcass
Day 0, control	3.16±0.24	27.16±2.24	12.28±1.47	1.30±0.08	56.10±2.18
Day 14					
Control	1.90±0.13	46.86±2.03	19.14±2.12	1.77±0.19	28.50±1.64
Medium	1.37±0.15*	50.70±5.47	14.10±2.44	1.87±0.54	28.82±4.59
High	0.77±0.13*	37.99±4.48	47.78±4.19*	0.74±0.16*	11.07±0.72*
Day 28					
Control	2.26±0.45	59.35±5.32	13.73±3.36	0.79±0.15	23.87±3.33
Medium	2.03±0.19	49.66±3.56	23.11±3.39	0.64±0.07	24.56±2.16
High	0.83±0.09*	51.00±3.91	35.05±3.89*	1.10±0.28	12.01±1.95*

Note: Statistical analysis was performed on arcsine-transformed proportional data.
*Significant difference ($p < 0.05$) from the respective control; $n = 30$ for day 0 control and $n = 10$ per tissue in all other treatments.

Fig. 2. Time course of waterborne ^{64}Cu uptake (means ± SEM) in previously unexposed rainbow trout on the control diet into the (A) gill, liver, gut, and carcass and (B) whole body ($n = 5$ per data point). Water Cu concentrations were 2.64 ± 0.21 ($n = 4$) and 2.81 ± 0.14 $\mu g \cdot L^{-1}$ ($n = 4$) before and after the flux, respectively.



high dietary Cu was the appearance of numerous mitochondria in the apical region of the enterocytes (Fig. 5C). A number of cells showed characteristics of apoptosis (Fig. 5D). Apoptotic cells were shrunken and possessed many electron-dense bodies and very few microvilli and mitochondria. The plasma membrane appeared to be intact and showed no signs of rupture as would occur in necrotic cells. In addition, the midintestine of fish exposed to elevated dietary Cu showed dramatic organellar changes marked by formation of numerous lamellated bodies (Figs. 6A and 6B). The lamellated bodies were composed of a whorl of circular membranous lamellae surrounding electron-dense granules and occurred in the cytoplasm, or within mitochondria and lysosomes. As well, cell renewal was evidenced by observation of mitosis and immature cells (Fig. 6C). Immature mucous cells contained extensive rough endoplasmic reticulum, a well-developed Golgi apparatus, and secretory granules at various stages of formation (Fig. 6C). In the lamina propria and the stratum compactum, there was an apparent increase in the number of eosinophilic granule cells (Fig 6D). The eosinophilic granule cells contained electron-dense granules of different sizes and occasional myelin bodies.

Discussion

Growth

Within the range of concentrations tested here, inhibitory growth effects of dietary Cu have been reported in rainbow trout (Lanno et al. 1985) and channel catfish (*Ictalurus punctatus*) (Murai et al. 1981), while lack of effect has been reported in channel catfish (Gatlin and Wilson 1986), rainbow trout (Mount et al. 1994; Handy et al. 1999), and Atlan-

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Fig. 3. (A) Relationship between body mass (x) and waterborne ^{64}Cu uptake (y) across the gills in previously unexposed rainbow trout on the control diet (n = 144). The relationship is best described by the negative exponential equation $y = 0.179 \exp(-0.0743x)$ (r^2 = 0.45, $p < 0.0001$). (B) Cu uptake rate measured with ^{64}Cu at different water Cu concentrations in unexposed fish (solid circles) and fish exposed to a high-Cu diet for 14 days (open circles). Values are means ± SEM, n = 5 per group per sampling time. The equation in Fig. 3A was used to correct for size differences; rates are expressed for 10-g fish. An asterisk indicates a significant difference.

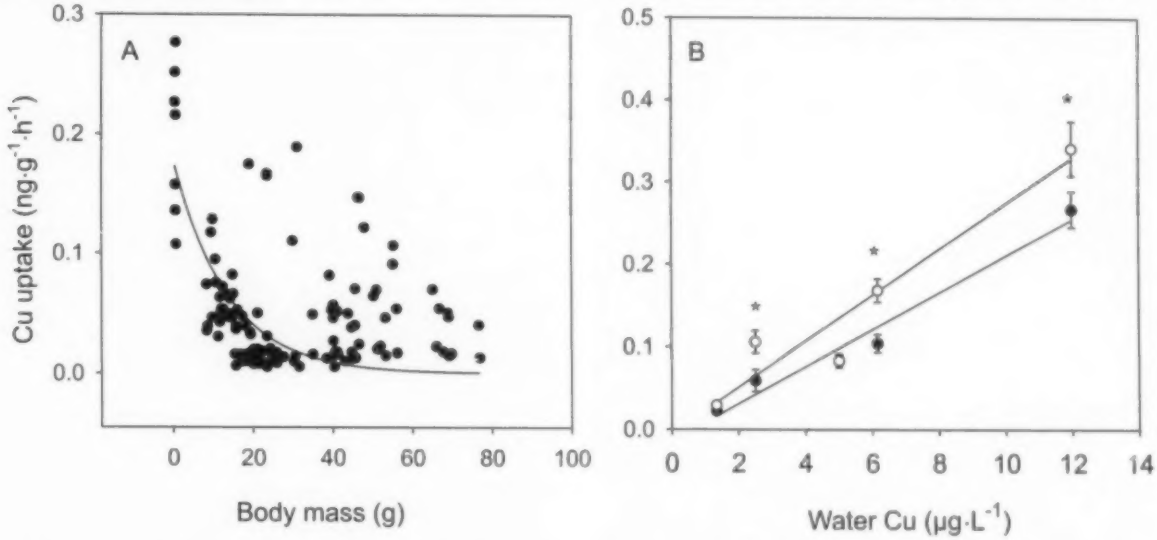
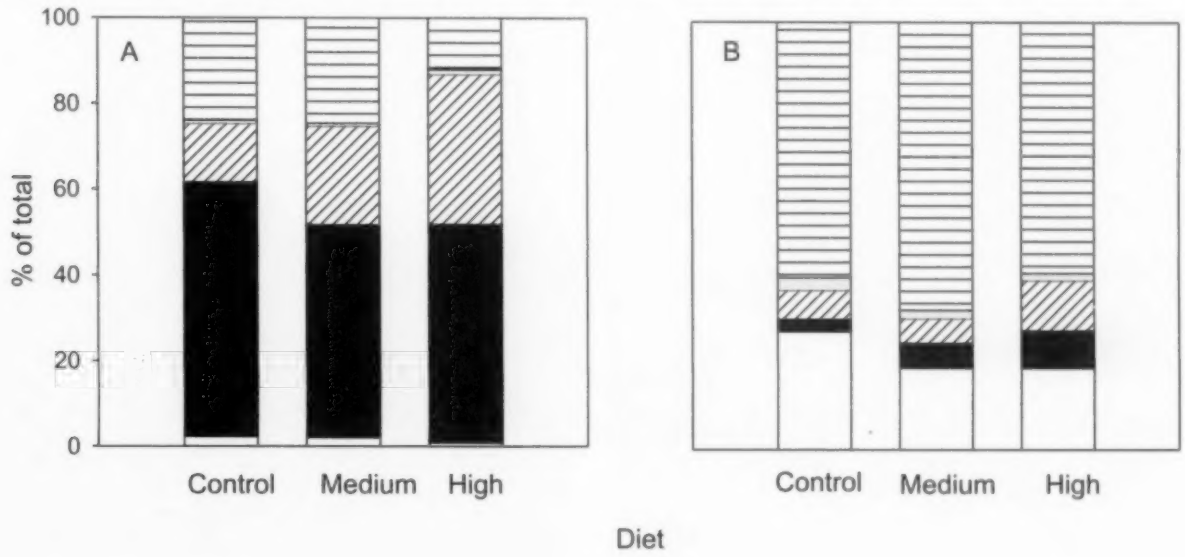


Fig. 4. Proportional distribution of (A) total Cu and (B) waterborne ^{64}Cu in the carcass (horizontal hatch), kidney (gray), gut (diagonal hatch), liver (solid), and gill (open) of control and dietary Cu exposed rainbow at day 28 (n = 10 per treatment).



tic salmon (Berntssen et al. 1999). Interestingly, Mount et al. (1994) reported mortalities in the absence of growth effect. In the present study, no significant growth effects or mortalities were manifest even in fish at a dietary Cu exposure as high as 1042 µg·g^{-1}. According to Lanno et al. (1985), dietary Cu concentrations of 664–730 µg·g^{-1} and above would cause poor food conversion and retarded growth in rainbow trout. Although the discrepancy among these data sets could be due to the different exposure periods, it is also possible that dietary Cu levels necessary to cause toxicity are much higher than previously thought. Furthermore, the amount of Cu absorbed, and hence effects, may depend on the bioavailability of the Cu in different food formulations.

Bioaccumulation and partitioning of Cu

Gut tissue Cu accumulation exhibited saturation with time and dose dependence. Seemingly, elevated dietary Cu level within the nontoxic range was well regulated. On a local ba-

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Table 4. Newly accumulated Cu (means ± SEM, ng·g wet weight^{-1} or μg·mL^{-1}, n = 30 for day 0 samples and n = 10 for day 14 and 28 samples).

	Gill (ng·g^{-1})	Liver (ng·g^{-1})	Gut (ng·g^{-1})	Kidney (ng·g^{-1})	Muscle (ng·g^{-1})	Carcass (ng·g^{-1})	Bile (μg·mL^{-1})	Plasma (μg·mL^{-1})
Day 0, control	8.14±0.43	284.33±78.60	89.14±49.79	35.25±13.96	82.11±40.83	15.37±5.06	33.34±9.74	1.48±0.59
Day 14								
Control	2.73±0.27	198.71±46.67	52.18±20.90	177.83±61.68	142.99±59.52	31.00±7.16	56.67±14.15	0.48±0.09
Medium	2.82±0.42	485.20±152.17	403.12±260.54	376.20±103.21	261.57±102.78	57.73±20.99	215.54±141.62	0.61±0.12
High	2.22±0.18	419.72±129.55	190.93±54.51*	233.05±58.52	183.76±62.25	37.81±11.05	139.23±39.47	1.10±0.35
Day 28								
Control	1.86±0.10	61.76±9.05	167.64±24.90	36.85±6.38	53.18±8.67	30.01±5.50	262.82±104.85	1.18±0.29
Medium	1.96±0.30	272.41±134.11	135.28±19.12	38.40±7.71	34.59±7.44	33.66±5.08	52.65±14.20	1.75±0.37
High	2.41±0.27	325.11±71.73*	177.07±31.50	170.74±88.98	32.44±5.55	45.77±6.71	132.06±45.54	1.59±0.26

*Significant difference ($p < 0.05$) from respective control.

Fig. 5. Representative electron micrographs of the apical region of the midintestine in control and dietary Cu exposed rainbow trout. (A) Control. E, enterocyte; L, lumen; Mv, microvilli; Ld, lipid droplet; m, mitochondrion; small arrowhead, tight junction; large arrowhead, desmosome. (B) Control. mc, mucous cell; sg, secretory granule. (C) Cu exposed. Arrows, mitochondria; arrowhead, multivesicular body. (D) Cu exposed. AE, apoptotic enterocyte; E, normal enterocytes; arrows, electron-dense granules in apoptotic cell; small arrowheads, multivesicular body; large arrowhead, denuded mirovillus.

sis, small but significant Cu accumulation occurred in gut tissue during exposure to the medium-Cu diet, and internally, increases in most tissue compartments were modest. Therefore, 300 μg Cu·g^{-1} in the diet appears to be well tolerated and may well be in the nutritional range, since it stimulated growth slightly and did not cause severe accumulation of Cu. In contrast, the high-Cu diet, representing a threefold increase in dietary Cu concentration relative to the medium-Cu diet, resulted in a dramatic 20-fold increase (relative to day 0) in gut tissue Cu concentration, a marked increase in Cu concentration in most internal compartments (especially the liver), and a nonsignificant tendency for reduced growth, suggesting that it was beyond the nutritional range.

The general trend at both levels of exposure was that during the early stage of exposure (14 days), a greater proportion of the Cu was retained in the gut tissue than at the later stage (day 28). This is probably due to the mobilization of the Cu from the primary site of uptake to other tissues, especially the liver. Furthermore, dietary Cu exposure resulted in continuous Cu accumulation in the whole body in contrast with waterborne Cu exposure where stable whole-body Cu was reported in rainbow trout (Marr et al. 1996; Taylor et al. 2000) following 1–2 months of waterborne Cu exposure. This probably indicates that dietary Cu uptake is less tightly controlled than waterborne Cu uptake.

Liver Cu accumulation was linear over time and dose dependent and was higher than that of all the other tissues, in agreement with previous studies (Julshamn et al. 1988; Handy 1993). The response of the liver to the 300 μg·g^{-1} diet is important with regard to the threshold of dietary Cu causing significant accumulation in the liver. Handy (1992) reported that dietary Cu of 200 μg·g^{-1} did not cause significant accumulation in rainbow trout liver. Results from this study show that 300 μg Cu·g^{-1} caused significant accumulation in the liver early in the exposure but not later in the exposure, indicating that regulatory mechanisms had checked further accumulation. It can therefore be deduced that the maximum dietary level of Cu causing sustained significant accumulation in juvenile rainbow trout liver at 4% body weight ration is greater than 300 μg·g^{-1}.

Copper concentration in the gills was significantly elevated at both dietary levels, in agreement with previous reports on exposure to sublethal dietary Cu for 32 days (Handy 1992) and 42 days (Miller et al. 1993). Accumulation of dietary Cu in the gill underscores the unique anatomical location of fish gills and their potential importance in Cu metabolism and toxicity. The gill Cu levels obtained in this study are comparable with those associated with death from waterborne Cu exposure (MacRae et al. 1999). Al-

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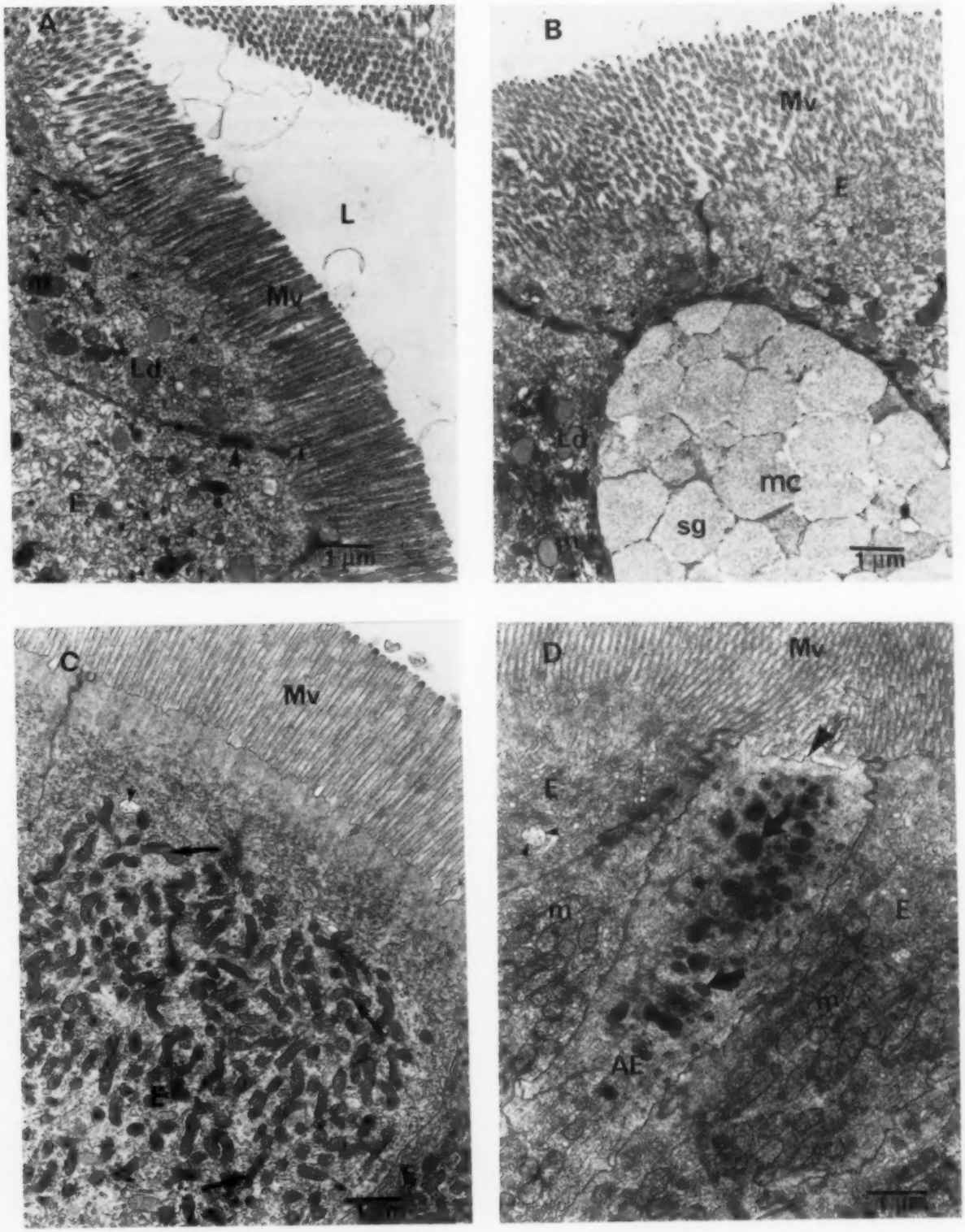

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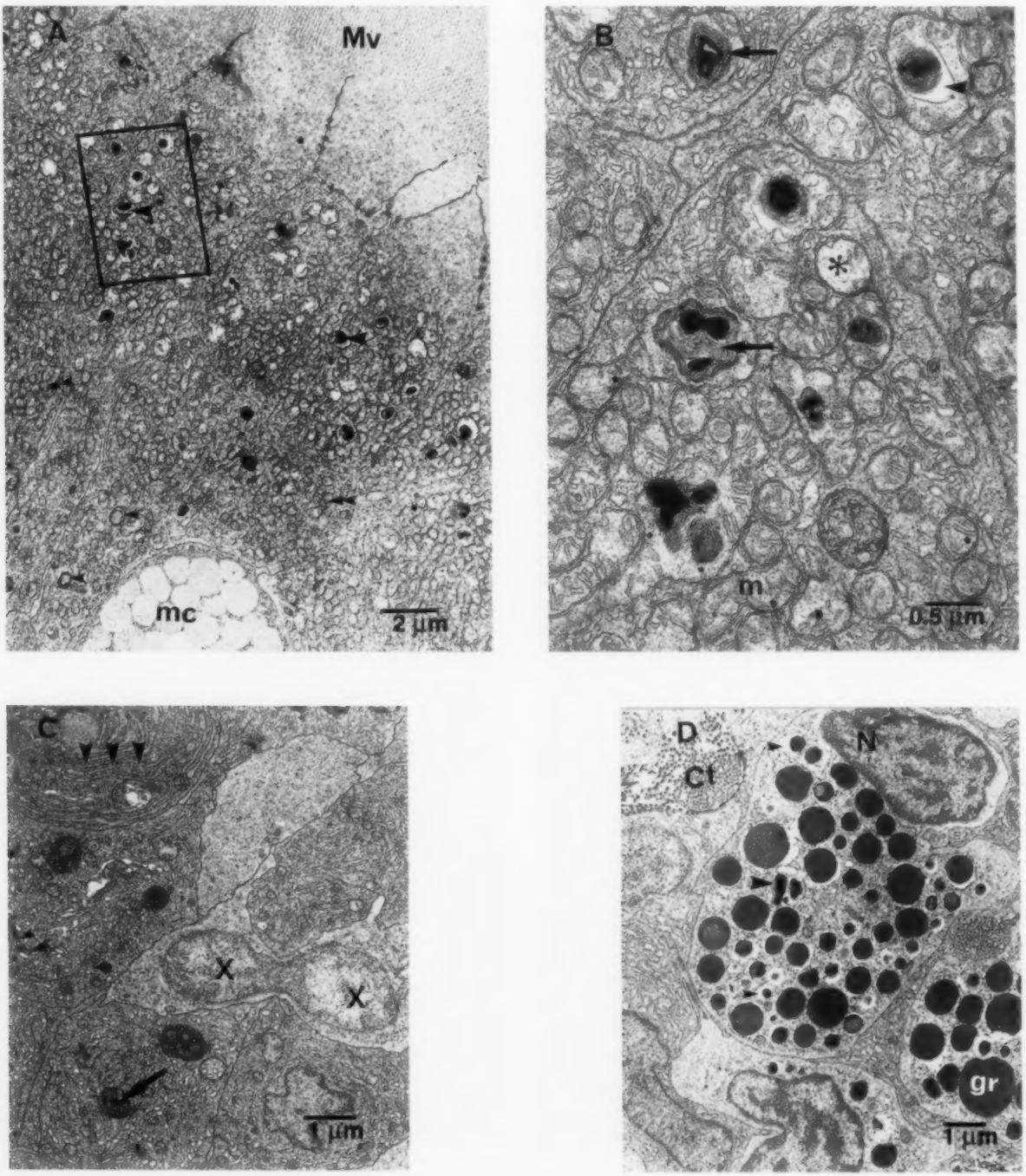
Fig. 6. Representative electron micrographs of enterocytes in a part of the midintestine of rainbow trout exposed to elevated dietary Cu. (A) Changes within enterocytes. Mv, microvilli on the apical side of an enterocyte; mc, mucous cell; large arrowheads, lamellated bodies; small arrowheads, secondary lysosomes. Note the numerous mitochondria (double arrowheads). (B) Closeup of the inset in Fig. 6A. Arrows, lamellated bodies with electron-dense granules; m, mitochondrion; asterisk, vacuole; arrowhead, halo. (C) Mitotic activity. Small arrowheads, Golgi apparatus; asterisk, newly formed secretory granule; large arrowheads, rough endoplasmic reticulum; X, dividing cell nucleus; arrow, secondary lysosome. (D) Eosinophilic granule cell. N, nucleus; gr, granule; large arrowhead, myelin body; small arrowheads, halo; Ct, connective tissue.



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though these authors reported that 1.4 μg Cu·g gill tissue^{-1} accumulated in 12 h caused 50% mortality in rainbow trout within 5 days, 1.37 μg Cu·g gill tissue^{-1} accumulated in 28 days of exposure to the high-Cu diet resulted in no mortality. It is possible that the majority of the Cu binding to gills during waterborne Cu exposure is the toxic Cu^{2+} species, while that accumulated in gills after dietary exposure is protein bound (after passage through the liver and basolateral membranes of the gill cells) and probably nontoxic.

Muscle Cu concentration was not affected by dietary exposure except on day 28 when fish exposed to the high dietary Cu showed a significant accumulation. Thus, muscle Cu concentration appears to be homeostatically regulated. Total plasma Cu levels also remained remarkably constant throughout the exposure, in agreement with previous studies (Miller et al. 1993; Berntssen et al. 1999). In one study where elevated plasma Cu was reported (Handy 1992), sampling was not preceded by a substantial period of starvation.

One of the regulatory responses associated with acclimation to waterborne Cu exposure in rainbow trout is increased hepatobiliary Cu excretion (Grosell et al. 1997, 1998). In the present study, increased biliary Cu concentration was observed at day 14, which remained elevated at the same level at day 28, indicating that a balance between Cu uptake into the liver and excretion into bile had been established. It would appear that biliary excretion of Cu, perhaps acting in concert with other regulatory mechanisms not assessed in this study, prevents serious changes in Cu distribution at a dietary Cu concentration of 300 μg·g^{-1}. However, the regulatory capacity is overcome at the high exposure dose (1000 μg·g^{-1}), resulting in Cu accumulation in the liver and other tissues.

Waterborne Cu uptake

Branchial uptake of waterborne Cu increased with fish mass, but when plotted on a per unit body mass basis, a negative exponential relationship was evident. When the allometric equation of Hughes (1984) was used to calculate gill surface area of the fish, it was clear that a difference in surface area explained only a small portion of the relationship. While Cu uptake per unit body weight changed approximately eightfold over the weight range, gill surface area changed by less than 1.5-fold. Thus, other factors are probably also involved. For example, variations in Cu transporter activities and metabolic rates among fish of different size could affect Cu uptake independent of gill surface area.

Absolute rates of Cu uptake will probably be affected by the speciation and therefore the bioavailability of Cu in the water. In the moderately hard, moderately alkaline water of our experiments, Cu largely exists as $Cu(OH)_x$ and $CuCO_3$, which are less bioavailable than Cu^{2+}. For example, Taylor et al. (2000) reported that Cu was more bioavailable to rainbow trout in moderately acidic soft water than in the present water quality.

In a previous study on the interaction between dietary and waterborne Cu, Miller et al. (1993) reported that the uptakes of dietary and waterborne Cu were independent. Our observations using a sensitive radioisotope methodology demonstrate that pre-exposure of Cu through the diet decreased uptake of waterborne Cu. Thus, we provide the first evidence of a homeostatic interaction between the two routes of uptake. Elevated body (including gill) Cu burden negatively influences Cu uptake, perhaps by occupying potential new Cu binding sites or causing down-regulation of Cu transport proteins, to check further increase in tissue Cu concentrations. The presence of a Cu ATPase in rainbow trout gill has recently been suggested (Campbell et al. 1999) based on inhibition of Cu uptake by serosal vanadate in perfused rainbow trout head preparations. Although the mechanisms of Cu uptake across the gills are yet to be clearly explained, previous Cu exposure appears to play some role in the regulatory mechanisms.

The newly accumulated Cu data indicate that the short-term exchangeable pool of Cu within the fish is very small relative to the total body Cu. In all the tissues sampled, except for the bile and plasma, the newly accumulated Cu was one to two orders of magnitude lower than the total Cu. However, in bile and plasma, the calculated values were higher than the total Cu. The apparent overestimation of newly accumulated Cu in the plasma and bile can be explained by high total Cu levels coupled with low specific activities in the previous compartments (gill and liver for plasma and bile, respectively) and lack of equilibrium between ^{64}Cu and total Cu pools. Such a scenario means that the ^{64}Cu is more available than total Cu for exchange in the plasma and bile. In this study, although ^{64}Cu activity was reduced in fish pre-exposed to dietary Cu, newly accumulated Cu in the tissues sampled (taking into account the dilution of ^{64}Cu within the branchial, plasma, and hepatic compartments) was not different among the treatment groups.

However, the distribution pattern of ^{64}Cu among various tissues differed greatly from that of total Cu. While the higher gill and lower gut incorporations were not surprising considering that ^{64}Cu uptake was measured from waterborne exposure, the very high carcass incorporation was remarkable. The carcass is mainly composed of white muscle, and Grosell et al. (2000) have recently shown that white muscle serves as an important short-term buffer compartment when trout are infused with radiolabeled Cu.

Midintestinal morphology

The morphology of the midintestine in control fish was similar to that described previously for normal rainbow trout (Ezeasor and Stokoe 1980; Nonnotte et al. 1986). The impact of dietary Cu on midintestinal structure could be categorized broadly into cellular and subcellular responses. The cellular response was characterized by an increase in apoptosis and cell division in the intestinal epithelium, similar to that reported in previous studies on Pb and Cd (Crespo et al. 1986) as well as on Cu (Berntssen et al. 1999). These changes are therefore not specific to Cu. Assuming that the dying epithelial cells have the same or higher Cu content than the normal ones, evacuation of these dead cells in feces or intestinal mucus would provide a significant route for excretion of dietary Cu. In addition, many of the immature cells were mucous cells, suggesting an increased need for mucus and its attendant protective role. Although metal-binding properties of gastrointestinal mucus have not been assessed in fish, mammalian studies have shown that intestinal mucus will strongly bind divalent ions (Colman and Young 1979).

The appearance of numerous lamellated bodies and multivesicular bodies in intestinal epithelium has not been previ-

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ously reported for dietary Cu exposure in fish. In mammalian studies, lamellated bodies are associated with regressive changes affecting cell organelles, e.g., in lysosomes during treatment with drugs and in mitochondria during exposure to toxic agents such as chloroform (Lüllmann-Rauch 1979; Guastadisegni et al. 1999). It is conceivable that abnormally high intracellular Cu causes damage and rearrangement of organelle membranes. The lysosomes observed in the enterocytes would possibly play a role in intestinal Cu sequestration and detoxification similar to their role in the liver during exposure to high Cu levels (Weiss et al. 1986; Lanno et al. 1987). The apparent increase in the number of mitochondria in midintestinal enterocytes suggests increased energy demand. It is possible that the uptake and (or) local regulation of Cu occur by energy-requiring processes and mitochondria supply the necessary energy to drive these processes. Conversely, Cu may impair energy-requiring activities in the enterocytes and the animal responds by increasing the number of mitochondria as a compensatory measure. Finally, dietary Cu stimulated an increase in eosinophilic granule cell infiltration in the lamina propria and stratum compactum. Eosinophilic granule cells have been hypothesized to constitute a composite defense mechanism, both humoral and mechanical, that develops in response to environmental demand (Ezeasor and Stokoe 1980).

The structural changes observed in the midintestine might be expected to have a metabolic cost, although only a marginal decrease in growth was evident at the high exposure dosage. More sensitive parameters, e.g., oxygen consumption or protein synthesis rates, might be more appropriate for assessing the cost of Cu exposure. Nonetheless, it is noteworthy that dietary Cu only modestly affected gut morphology. The bioaccumulation data indicate that, unlike the other tissues, gut tissue Cu was regulated and concentration stabilized by day 14. The absence of growth effect suggests that nutrient absorption in the gut was not significantly compromised, a persuasive indication that dietary Cu did not severely impact gut structure and function.

In conclusion, rainbow trout regulated dietary Cu at the level of the gut by increasing clearance to other tissues, at the liver by increasing biliary Cu excretion, and at the gill by reducing waterborne Cu uptake in response to dietary exposure. The modest morphological changes in the intestinal tract suggested high cell and organelle turnover and local regulation of Cu. In spite of possible increased energy demand for regulation and tissue repair, there was no significant growth inhibitory effect.

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Does spawning stock affect recruitment of New England groundfish?

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Abstract: We evaluated the effects of spawning-stock size on the recruitment abundance of 11 groundfish stocks in waters off New England. Empirical patterns of association between spawning biomass and recruitment were investigated using nonparametric approaches. For most stocks, higher recruitment occurred at high stock sizes and lower recruitment at low stock sizes. Survival ratios, indexed by recruitment per unit of spawning biomass, were higher at low stock sizes and lower at high stock sizes—as expected under density dependence. To assess assumptions about recruitment dynamics, we analyzed constant-recruitment and compensatory and overcompensatory stock–recruitment models with uncorrelated and autocorrelated error structures, using Bayesian methods of statistical inference. Although no single model was adequate for all stocks, most groundfish had better than even odds of having density-dependent dynamics, and most had better than even odds of having dynamics with uncorrelated environmental variation. While some geographic and taxonomic differences in potential productivity were apparent, all stocks exhibited compensation in survival ratio at low stock sizes, albeit with substantial variation. These results indicate that conservation measures intended to increase the spawning biomass of New England groundfish will, on average, result in higher recruitment and, eventually, in increased and more stable fishery yields.

Résumé : Nous avons examiné les effets de la densité des reproducteurs sur l'importance du recrutement chez 11 stocks de poissons benthiques au large de la Nouvelle-Angleterre. Les patterns empiriques d'association entre la biomasse des reproducteurs et le recrutement ont été étudiés à l'aide de méthodes non-paramétriques. Pour la plupart des stocks, il y a un recrutement plus élevé lorsque la densité des reproducteurs est grande, et un recrutement faible lorsque la densité est faible. Les rapports de survie, exprimés comme le recrutement par unité de biomasse des reproducteurs, sont plus élevés lorsque la densité des reproducteurs est faible et plus bas lorsque la densité est élevée—comme cela arrive dans les cas de densité-dépendance. Pour évaluer diverses suppositions sur la dynamique du recrutement, nous avons analysé, à l'aide de méthodes bayésiennes d'inférence statistique, des modèles reliant la densité des reproducteurs et le recrutement, modèles de type constant, compensatoire et surcompensatoire, avec des structures d'erreur non-corrélées et autocorrélées. Bien qu'aucun modèle particulier ne se soit montré adéquat pour tous les stocks, la plupart des stocks ont une probabilité plus qu'aléatoire d'avoir une dynamique densité-dépendante et cette dynamique tend à n'être pas corrélée à l'environnement. Malgré certaines différences géographiques et taxonomiques dans la productivité potentielle, tous les stocks montrent de la compensation, bien qu'avec beaucoup de variation, dans le rapport de survie lorsque les densités des reproducteurs sont basses. Ces données indiquent que les mesures de conservation destinées à accroître la biomasse des reproducteurs chez les poissons benthiques de la Nouvelle-Angleterre résulteront, en moyenne, en un recrutement plus élevé et, éventuellement, en des rendements de pêche plus grands et plus stables.

[Traduit par la Rédaction]

Introduction

Competing theories on whether climate, spawner abundance, or trophic interactions are the primary determinants of marine fish stock recruitment have been debated for decades. The mechanisms underlying recruitment variability have great practical significance for fishery management. Two dichotomous views are that either population-level (density-dependent) processes or environmental (density-independent) processes primarily determine recruitment strength. While neither density-dependent nor density-independent regulation will explain recruitment variability in all cases, each has its proponents (Laevastu and Favorite 1988; Myers and Barrowman 1996 and references therein), and debate continues over which theory is best supported (Gilbert 1997; Hilborn 1997; Myers 1997). The relative importance of density-dependent versus density-independent processes is contingent upon species and ecosystem. In practice, quantifying the relative importance of each must be done on a case-by-case basis. In this context, we determined the relative importance of density-dependent and density-independent processes on recruitment of 11 commercially valuable groundfish stocks off the New England coast.

Many New England groundfish stocks have been overfished by distant-water and U.S. fleets over the past three decades (Murawski et al. 1997). Under provisions of the U.S.

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Sustainable Fisheries Act of 1996, rebuilding plans must be developed and implemented for all overfished resources within U.S. waters. The question of how rapidly stocks can be rebuilt depends, in large part, upon future recruitment. Because future recruitment will fluctuate, stochastic models of stock–recruitment relationships are essential to meaningfully evaluate rebuilding trajectories for depleted stocks. To this end, stock–recruitment data for 11 groundfish stocks in the Gulf of Maine, Georges Bank, and southern New England areas (Fig. 1) were used to analyze assumptions about stock–recruitment dynamics. These stocks included: Georges Bank haddock (*Melanogrammus aeglefinus*); Georges Bank and Gulf of Maine cod (*Gadus morhua*); Georges Bank, southern New England, and Gulf of Maine yellowtail flounder (*Limanda ferruginea*); Georges Bank and southern New England winter flounder (*Pseudopleuronectes americanus*); Gulf of Maine witch flounder (*Glyptocephalus cynoglossus*); Gulf of Maine American plaice (*Hippoglossoides platessoides*); and Gulf of Maine white hake (*Urophycis tenuis*).

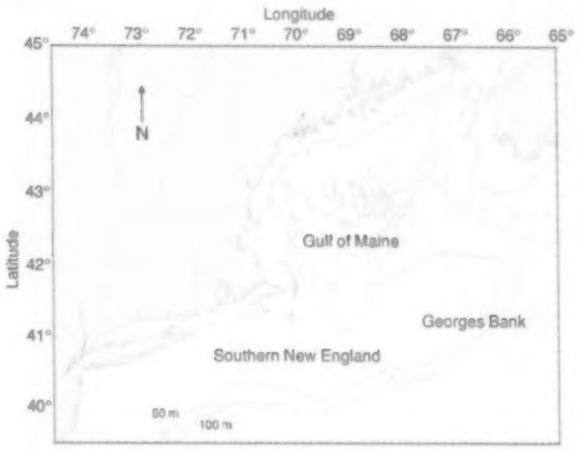
To analyze these data, we first enlarged our stock–recruitment data sets by extending the available time series backwards to include years not covered by sequential age-structured population analyses by relating absolute-population estimates to relative abundance indices from fishery-independent surveys. Empirical relationships in the enlarged data sets were analyzed to detect compensation (e.g., higher recruitment per spawner at low spawner abundance) and temporal trends in recruitment (R), spawner abundance (S), and recruitment per spawner ratios (R/S). To assess assumptions about recruitment dynamics, we analyzed six compensatory stock–recruitment models: constant recruitment, compensatory, and overcompensatory (e.g., lower R at very high S), with uncorrelated and serially correlated random variation. The appropriateness of stock–recruitment models was assessed using a small-sample form of Akaike's information criterion (AIC_C; Burnham and Anderson 1998). We calculated the posterior probability of each stock–recruitment hypothesis from its relative AIC_C, using Bayes' theorem with an uninformative prior. These probabilities quantified the stock–recruitment model uncertainty for each of the 11 New England groundfish stocks. We also investigated variations in potential productivity by subarea and taxonomic group.

Materials and methods

Stock–recruitment data

For each of the 11 stocks, age-structured assessments were recently updated to assess stock status and provide management information (Northeast Fisheries Science Center 1999), using a sequential population analysis (SPA) model (ADAPT; Gavaris 1988; Conser and Powers 1990; National Research Council 1998). The ADAPT formulation uses age-specific abundance indices from research surveys to calibrate SPA estimates of stock size and fishing mortality. Estimates of population numbers at age from this SPA model were limited in time by the availability of catch-at-age estimates. As a result, each series of SPA-based stock-size estimates was much shorter than the full time series of fishery-independent abundance data available from the autumn groundfish bottom trawl survey conducted by the Northeast Fisheries Science Center (NEFSC; Azarovitz 1981) from 1963 onwards, except for Georges Bank haddock (Table 1). To extend and enlarge the time series of stock–recruitment data, we used the estimated catchability

Fig. 1. New England groundfish stock areas in the northwest Atlantic.



coefficients from the SPA, $q = n \cdot N^{-1}$, relating the survey abundance index n to the population size N estimated by SPA, to calculate recruitment and spawning-stock sizes from the 1960s to the present. This approach had been previously used to extend the time series of stock–recruitment data for Gulf of Maine cod (National Research Council 1998).

Estimates of catchability at age (q_a) for the NEFSC autumn survey from the age-structured assessment were used to produce estimates of time series of recruitment for each stock, except for Georges Bank haddock. We defined the age of recruitment and its associated length range for each stock on the basis of age at capture in the commercial fishery. In some cases, this led to an older age of recruitment than was used in the assessment (typically, age-1 or age-2). Given the index of recruitment from the autumn survey during the yth year, $I(y)$, we computed recruitment in year y as the survey recruitment index divided by the age-specific catchability, $R(y) = I(y) \cdot q_a^{-1}$. Using survey data to measure recruitment may be less susceptible to errors in catch at age (owing to discarding or under-reporting of the catch) than SPA-based estimates (Myers 1998), but may also result in substantial measurement error, because less information is used to estimate stock size.

Estimates of survey catchability at age were also used to calculate spawning stock. The procedure that was used depended on the availability of survey age data. For the four stocks with age-specific survey time series for 1963–1998 (Georges Bank cod and yellowtail flounder; southern New England yellowtail flounder; and Gulf of Maine cod), age-specific catchabilities were directly applied to survey indices. Spawning biomass in year y ($S(y)$), given the average weight of an age-a spawner in year y ($W_a(y)$), was

$$(1) \qquad S(y) = \sum_a = \frac{I_a(y) W_a(y)}{q_a(y)}$$

For the six stocks lacking historical-survey age data (Gulf of Maine witch flounder, American plaice, white hake, and yellowtail flounder; and Georges Bank and southern New England winter flounder) but possessing length-composition data, a length cutoff representing the onset of full maturity at the time of survey was applied. Survey numbers at length were summed from the cutoff length to the maximum length and the mean length was calculated. Mean weights were converted from mean lengths using the length–weight equation for each stock. An estimate of average autumn survey catchability of fully mature age-classes ($\bar{q}_s$) was computed as the average of survey catchabilities at age weighted by ADAPT

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Table 1. Summary of stock–recruitment time series, Pearson correlation coefficients between estimated spawning biomass (S) and recruitment (R) with sequential population analysis (SPA) estimates, and maximum observed spawning biomass (S_{MAX}, millions of kilograms) for 11 New England groundfish stocks.

						Correlations		
Stock	Scientific name	SPA series	Estimated series	Spawner ages	Recruitment age	S	R	S_{MAX}
Gulf of Maine								
American plaice	*Hippoglossoides platessoides*	1980–1996	1963–1994	Age-3+	Age-2	0.80***	0.87***	20.9
Witch flounder	*Glyptocephalus cynoglossus*	1982–1994	1963–1995	Age-4+	Age-3	0.69**	0.78***	128.4
Cod	*Gadus morhua*	1982–1996	1963–1996	Age-2+	Age-2	0.66***	0.88***	85.7
White hake	*Urophycis tenuis*	1985–1996	1963–1995	Age-3+	Age-2	0.70**	0.86***	34.0
Yellowtail flounder	*Limanda ferruginea*	1985–1997	1979–1996	Age-2+	Age-2	0.48*	0.45*	2.4
Georges Bank								
Haddock	*Melanogrammus aeglefinus*	1931–1998	—	Age-2+	Age-1	—	—	199.5
Cod	*Gadus morhua*	1978–1998	1963–1996	Age-2+	Age-2	0.48**	0.54**	233.8
Yellowtail flounder	*Limanda ferruginea*	1973–1997	1963–1996	Age-2+	Age-2	0.85***	0.50**	46.4
Winter flounder	*Pseudopleuronectes americanus*	1982–1996	1963–1995	Age-3+	Age-2	0.63**	0.39*	18.5
Southern New England								
Winter flounder	*Pseudopleuronectes americanus*	1981–1997	1963–1996	Age-3+	Age-2	0.57**	0.64***	48.8
Yellowtail flounder	*Limanda ferruginea*	1973–1997	1963–1996	Age-2+	Age-2	0.76***	0.82***	122.9

Note: *, $0.20 \geq P > 0.05$; **, $0.05 \geq P > 0.01$; ***, $P \leq 0.01$.

estimates of numbers at age for the terminal year of the sequential population analysis (N_a):

$$(2) \qquad \bar{q}_S = \frac{\sum_a q_a N_a}{\sum_a N_a}$$

Spawning-stock biomass was the sum of fully mature age-classes of survey numbers at length ($I_L(y)$) multiplied by the average autumn survey weight at length ($W_L(y)$) divided by the average spawner catchability as

$$(3) \qquad S(y) = \frac{\sum_L W_L(y) I_L(y)}{\bar{q}_s}$$

Length frequency information was inadequate to perform these calculations for one or two of the years for Gulf of Maine witch flounder (1984 and 1985), Georges Bank winter flounder (1989), and southern New England winter flounder (1966 and 1969).

We compared estimated time series of spawning biomass and recruitment with the ADAPT time series where the two series overlapped (Table 1). The two time series of recruitments were positively correlated, with Pearson correlation coefficients ranging from 0.39 to 0.88 (Table 1): an example for recruitment of Gulf of Maine witch flounder shows a typical pattern (Fig. 2*a*). Similarly, the SPA and imputed series of spawning stock size estimates were highly positively correlated, with correlations ranging from 0.48 to 0.85: an example for spawning biomass of Gulf of Maine witch flounder shows a typical pattern (Fig. 2*b*). By using the catchability-based estimates, we added 141 stock–recruitment data points. With the exception of Gulf of Maine American plaice, these additional data provided greater contrast in recruitment and spawning-stock sizes.

Empirical patterns

Empirical patterns of association between spawning biomass and recruitment were investigated using nonparametric approaches. The relative ranks of maximum and minimum recruitment ranked by the spawning biomass that produced them (see Myers and Barrowman 1996) were calculated, to show whether extreme values of R had been produced at relatively high (rank near 1) or low (rank near 0) spawning biomass. Similarly, the relative ranks of maximum- and minimum-survival ratios (recruitment per unit of spawner biomass (R/S)) ranked by spawning biomass and the ratios of expected R/S above median spawner abundance ($E[(R/S)_{S>\text{med}(S)}]$) to expected R/S below median spawner abundance ($E[(R/S)_{S<\text{med}(S)}]$) were calculated for each stock, to show whether survival ratios depended upon spawning-stock size. In theory, R/S values would be expected to decline as spawning stock increased and to increase as spawning stock declined, in the absence of depensation. To detect long-term trends in recruitment and spawning stock, linear regressions of R versus time and S versus time were computed. For comparison, temporal fluctuations in survival ratios were discerned using a locally weighted scatterplot smoother (Cleveland 1979) with a tension parameter of ½. The significance of the relative rankings, ratios, and regression slopes were tested using randomization methods (Manly 1997). To do this, the randomization distribution was computed for each statistic (e.g., for the relative rank, ratio, or slope), using 5000 random permutations of the input data. The observed statistic was then compared with its randomized distribution, to assign probability values of obtaining a more extreme outcome by chance.

To detect association between recruitment and spawning-stock size, we applied log-linear models (e.g., Bishop et al. 1975) to compute the odds of increased recruitment given an increase in

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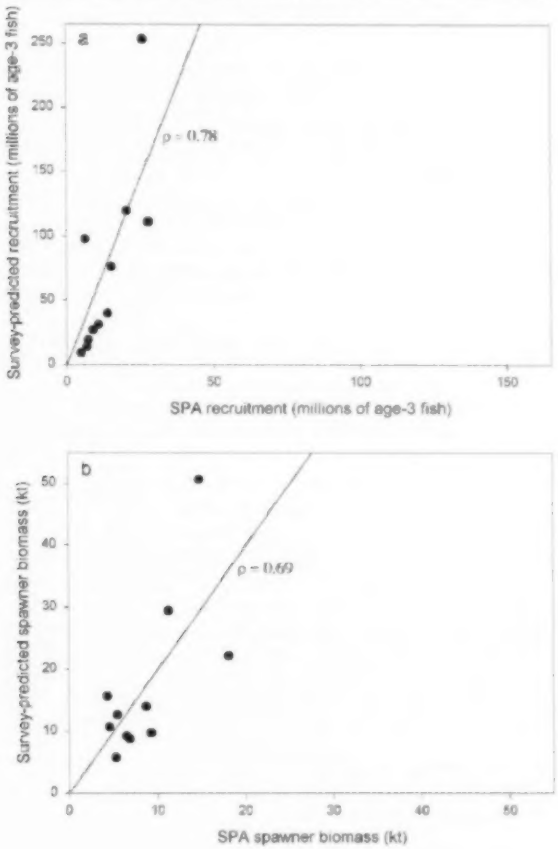
Fig. 2. Comparisons of sequential population analysis (SPA) estimates and survey-based estimates of recruitment (*a*) and spawner biomass (*b*) for witch flounder along with estimated correlations (ρ).



spawning biomass. Recruitment and spawning biomass time series were divided into groups (low, high) corresponding to observations below and above the median: an example for Georges Bank haddock shows the typical pattern of high variability in recruitment both above and below the median spawning biomass (Fig. 3). The association between recruitment and spawning biomass was summarized by a 2 × 2 contingency table. The response variable ($n_{S,R}$) was the number of observations (i.e., years) for each combination of the two levels of R and S, where index values of 1 and 2 indicated observations below and above the median, respectively. The odds that R and S coincided were determined from the 2 × 2 contingency table. For example, the conditional probability of attaining a high R when S was high was $n_{22} \cdot (n_{21} + n_{22})^{-1}$. Given the conditional probabilities, the odds (Ω) of attaining a high versus low R when S was above its median was

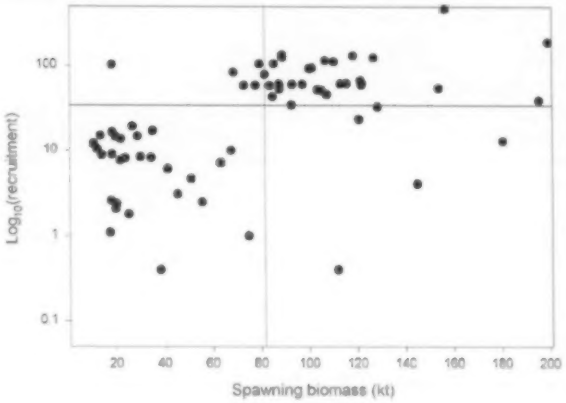
$$(4) \qquad \Omega_{S_{\text{HIGH}}} = \frac{\Pr(R_{\text{HIGH}} | S_{\text{HIGH}})}{\Pr(R_{\text{LOW}} | S_{\text{HIGH}})} = \frac{n_{22}}{n_{21}}$$

while the odds of attaining a high R versus low R when S was below its median was

$$(5) \qquad \Omega_{S_{\text{LOW}}} = \frac{\Pr(R_{\text{HIGH}} | S_{\text{LOW}})}{\Pr(R_{\text{LOW}} | S_{\text{LOW}})} = \frac{n_{12}}{n_{11}}$$

The odds ratio, denoted as O, was

Fig. 3. Scatterplot of Georges Bank haddock recruitment and spawning biomass in relation to median recruitment (horizontal line) and median spawning biomass (vertical line), 1931–1998.



$$(6) \qquad O = \frac{\Omega_{S_{\text{HIGH}}}}{\Omega_{S_{\text{LOW}}}} = \frac{n_{11} n_{22}}{n_{12} n_{21}}$$

An odds ratio equal to Q indicated that the odds of obtaining R above the median when S was high was Q times greater than the odds of obtaining R above the median when S was low. By symmetry, O^{-1} was the odds ratio of obtaining R above the median when S was low. Statistical significance of the odds ratio was assessed using the standard χ^2 statistic (which is reasonable when the number of observations exceeds the number of cells by a factor of from four to five). The expected value of recruitment when S was above or below the median was estimated as the geometric mean of the recruitment. The ratio of the geometric mean R at high and low S (recruitment ratio) was used to measure the expected change in recruitment from low to high spawning-stock size.

Stock–recruitment dynamics

We chose a set of models of stock–recruitment dynamics that was consistent with the empirical patterns. This indicated that spawning stock and recruitment were strongly correlated for some stocks, implying that modeling density dependence was important, and that recruitment variability was substantial across stocks; hence, modeling apparent random variation was important. Each model had a deterministic component accounting for compensation and a stochastic component accounting for environmental variability.

The deterministic component was either a density-independent, a compensatory, or an overcompensatory model. The density-independent model was $R = \mu_R$, where μ_R was mean recruitment for the observed time series. The compensatory model was a modified Beverton–Holt curve (Mace and Doonan 1988), with R related to S as

$$(7) \qquad R = \frac{4zR_{\text{MAX}}S}{S_{\text{MAX}}(1 - z) + S(5z - 1)}$$

where S_{MAX} is the maximum observed level in the stock–recruitment data; R_{MAX} is the maximum expected recruitment at a spawning-stock size of S_{MAX}; and z is the steepness of the modified Beverton–Holt curve computed as the ratio of R at 20% of S_{MAX} to R_{MAX}. The overcompensatory model was a Ricker curve:

$$(8) \qquad R = Se^{\alpha + \beta S}$$

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where α is the slope at the origin and β is the strength of density dependence in the relationship. The constant-recruitment model represented unbounded compensation as spawner abundance decreased, while the Beverton–Holt and Ricker curves represented the extremes of density-dependent behavior; recruitment either approaches an asymptote or decreases with spawning stock.

The stochastic component was represented by a multiplicative lognormal or an autoregressive multiplicative lognormal error structure with a lag of 1 year. The stochastic component was multiplied by the deterministic component, denoted as $f(S_i)$ for the ith data point, to obtain the stock–recruitment model:

$$(9) \qquad \hat{R}_i = f(S_i)e^{\varepsilon_i}$$

For uncorrelated errors, the ε_i were iid Gaussian random variables with zero mean and constant variance σ^2. In this case, the error variance (σ^2) was a parameter to be estimated. For autoregressive lag-1 errors, the ε_i were distributed as

$$(10) \qquad \varepsilon_i = \phi\varepsilon_{i-1} + w_i$$

where $|\phi| < 1$, $\mathrm{Var}(\varepsilon) = \sigma^2$, $w_i \sim N(0, \sigma_w^2)$, and $\sigma_w^2 = (1 - \phi^2)\sigma^2$ and the autoregressive coefficient and the error variance were additional model parameters to be estimated. We chose a multiplicative lognormal error term, because this positively skewed distribution arises naturally when groundfish survival rates during early life history are affected by numerous independent random events. The autoregressive error term was included to model serial correlation in random environmental variation. This choice of error structure allowed successive recruitments to be correlated when the effects of environmental forcing were strong—for example, periods of good recruitment followed by periods of poor recruitment—regardless of the deterministic component. Overall, this led to six possible models with a wide range of possible dynamical behavior.

The support functions, or loglikelihoods (log L), for a total of n stock–recruitment data points (R_i, S_i) with uncorrelated lognormal errors (eq. 11) and autoregressive lag-1 correlated lognormal errors (eq. 12) (see Seber and Wild 1989) were

$$(11) \qquad \log L(\theta, \sigma^2) = -\frac{n}{2}\log(2\pi) - n\log\sigma - \sum_{i=1}^{n}\log R_i - \frac{1}{2\sigma^2}\sum_{i=1}^{n}(\log R_i - \log f(S_i))^2$$

$$(12) \qquad \log L(\theta, \sigma^2, \phi) = -\frac{n}{2}\log(2\pi) - n\log\sigma_w - \sum_{i=1}^{n}\log R_i - \frac{1}{2}\log(1-\phi^2) - \frac{1}{2\sigma_w^2}\sum_{i=2}^{n}(\log R_i - \phi\log R_{i-1} - \log f(S_i) + \phi\log f(S_{i-1}))^2 - \frac{(1-\phi^2)}{2\sigma_w^2}(\log R_1 - \log f(S_1))^2$$

Maximum likelihood estimates (MLEs) of model parameters were computed using these support functions and the estimated time series of stock–recruitment data (Table 1). For stocks with gaps in stock–recruitment data, we used the longest continuous time series, in order to fit the autoregressive models. In particular, time series for estimating stock–recruitment models for Gulf of Maine witch flounder, Georges Bank winter flounder, and southern New England winter flounder were limited to 1963–1983, 1963–1988, and 1970–1996, respectively.

Likelihood-based selection criteria were applied to evaluate candidate models using two approaches. In the standard approach, we applied AIC_C (Burnham and Anderson 1998 and references therein), a bias-corrected form of AIC, to select a single best model. The value of AIC_C for a model fit to data set D, with K parameters, n data points, and likelihood value $L(D|\underline{\Theta})$ at the MLE $\underline{\Theta}$, equals

$$(13) \qquad AIC_C = -2\log L(D|\underline{\Theta}) + 2K + \frac{2K(K+1)}{n-K-1}$$

The best model had the lowest AIC_C value. However, when AIC_C values were very similar among models, support for a single best model was clearly limited.

Alternatively, Bayes' theorem was applied to evaluate the relative performance of each model. We computed the probability that each candidate model was correct given the available data. Estimated AIC_C values were used to measure the relative likelihood of each model, with a penalty applied for the number of parameters that differed, according to the assumed error structure, viz., let $\underline{M} = \{M_k\}$ denote the set of candidate models and let M_{MAX} denote the model with the maximum AIC_C value; M_{MAX} is the least likely model in $\underline{M}$. Thus, for a given set of stock–recruitment data D and model M with a corresponding AIC_C value of $AIC_C(D|M)$, the likelihood ratio of model M to the least likely model is $\Lambda(D|M, M_{MAX})$ where

$$(14) \qquad \Lambda(D|M, M_{MAX}) = \frac{L(D|\underline{\Theta}_M)}{L(D|\underline{\Theta}_{M_{MAX}})} \propto \frac{e^{-\frac{1}{2}AIC_C(D|M)}}{e^{-\frac{1}{2}AIC_C(D|M_{MAX})}}$$

The posterior distribution of relative model credibility was calculated from the likelihood ratio form of Bayes' theorem, using the model likelihood ratios relative to the least likely model and the prior distribution of each model, $\Pr(M_k)$. The posterior probability of model M, denoted by $\Pr(M|D)$, is the product of its likelihood ratio and prior probability divided by a normalizing constant:

$$(15) \qquad \Pr(M|D) = \frac{\Lambda(D|M, M_{MAX})\Pr(M)}{\sum_{M_k \in \underline{M}}\Lambda(D|M_k, M_{MAX})\Pr(M_k)}$$

In the absence of any prior information on the credibility of candidate models, we assumed equal prior probabilities for each of the six possible models. Given the posterior model probabilities, the expected value of the autoregressive parameter, $E[\phi]$, was computed for each stock, to show the strength of serial correlation in the stock–recruitment data. Similarly, the posterior odds ratio of density-dependent (Beverton–Holt or Ricker curves) to density-independent (constant-recruitment) models and the posterior odds ratio of uncorrelated to serially correlated environmental variation were computed for each stock.

To evaluate the strength of regional or taxonomic patterns in potential productivity, model results were compared among areas and related species. Regional comparisons were made by grouping stocks into two areas: (1) Georges Bank (haddock, cod, yellowtail flounder, and winter flounder) and southern New England (yellowtail flounder and winter flounder) and (2) the Gulf of Maine (cod, witch flounder, American plaice, yellowtail flounder, and white hake). Taxonomic groups included gadids (Georges Bank haddock and cod; and Gulf of Maine cod and white hake) and flounders (Georges Bank yellowtail and winter flounders; Gulf of Maine witch flounder, American plaice, and yellowtail flounder; and southern New England yellowtail and winter flounders). To contrast productivity of recruitment per unit area among groups, average group values were calculated for the expected ratio of recruitment at maximum observed spawning biomass to size of the mean area occupied by the stock ($R(S_{MAX})$/Area). For each stock, the expected values of these ratios were computed, using the MLEs of $R(S_{MAX})$ and the model probabilities $\Pr(M|D)$.

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Results

Empirical patterns

Recruitment variability was substantial for all 11 stocks. Coefficients of variation (CV = 100·SD·mean^{-1}) of R ranged from 59 to 133%. The relative rank of the S that produced the maximum recruitment (max(R)) in the spawning biomass time series was in the upper half of the data for 7 of the 11 stocks, and the relative rank of max(R) for Georges Bank haddock was significant (Table 2). Similarly, the relative rank of the S in the spawning biomass time series that produced the minimum recruitment (min(R)) was in the lower half of the data for 7 of the 11 stocks. Thus, despite substantial variability, the highest recruitments were generally produced at higher spawning biomasses and vice versa.

Variability in survival ratios was even greater than for recruitment. Coefficients of variation of R/S ranged from 69 to 232%, with most CVs near or above 100%. For all 11 stocks, the relative rank of the spawning stock that produced the maximum R/S value was below the median (Table 2). Gulf of Maine witch flounder and southern New England yellowtail flounder had significant relative ranks of max(R/S). Thus, per capita production of recruits peaked when spawning biomass was below the median. Similarly, the minimum R/S value occurred when spawning stock was above the median for 9 of the 11 stocks, and the relative rank of min(R/S) was significant for 6 of the 11 stocks. These patterns were consistent with expected compensation in stock–recruitment dynamics at higher stock sizes. The ratio of average R/S when S was above its median to the average R/S when S was below its median was less than unity for 10 of the 11 stocks and was significantly less than unity for 8 of the 11 stocks. Thus, survival ratios were, on average, lower at high spawning biomass and higher at low spawning biomass, as would be expected if density-dependent mechanisms were important.

Long-term declines in spawning stock and recruitment were detected for several stocks. Linear-regression analyses (Table 2) showed that 9 of the 11 stocks had a significant declining trend in spawning-stock biomass (e.g., Georges Bank haddock, cod, and yellowtail and winter flounders; Gulf of Maine cod, witch flounder, and American plaice; and southern New England yellowtail and winter flounders). The remaining two stocks, Gulf of Maine white hake and Gulf of Maine yellowtail flounder, had no time trend in spawning stock. Recruitment showed a significant declining trend for four stocks (Table 2): Georges Bank haddock and yellowtail flounder and southern New England yellowtail and winter flounders. One stock, Gulf of Maine witch flounder, had a significant increasing trend in recruitment. The remaining six stocks had no apparent trend in recruitment. Overall, the majority of stocks have experienced long-term declines in spawning stock, while long-term declines in recruitment were apparent in about one-third of the stocks.

Scatterplots of R/S values (with plot symbols proportional to annual recruitment) and locally weighted regression lines showed temporal changes in survival ratios for several stocks. In the Gulf of Maine, survival ratio (R/S) and recruitment trends varied by stock. For Gulf of Maine American plaice (Fig. 4*a*), R/S generally increased from 1962 onwards, with recruitment markedly increasing in the late 1980s. For Gulf of Maine witch flounder (Fig. 4*b*), R/S and recruitment were relatively stable during 1960–1980 but, subsequently, have substantially increased. In contrast, the survival ratio for Gulf of Maine cod (Fig. 4*c*) increased during 1963–1980 and has since declined. Recruitment for this stock was relatively high during the 1970s but has been much lower in recent years. For Gulf of Maine white hake (Fig. 4*d*), R/S values decreased during the period from 1960 to the mid-1970s, increased from the mid-1970s to the mid-1980s, and have since declined. Recruitment in this stock has increased since about 1980. For Gulf of Maine yellowtail flounder (Fig. 4*e*), survival ratios were relatively low and constant during 1980–1985 and 1990–1995 but were higher during the late 1980s. Recruitment was relatively high during the mid-1980s to early 1990s. Overall, survival ratios of the two Gulf of Maine gadid stocks have decreased in recent years, while survival ratios of two out of three flounder stocks have increased.

Within the continental shelf waters of Georges Bank and southern New England, haddock (Fig. 4*f*) had above-average R/S values, high spawning-stock sizes, and high recruitments during 1930–1960. Survival ratios then declined moderately from the mid-1960s to the mid-1970s, with marked declines in recruitment and spawning stock. Subsequently, both recruitment and R/S have moderately increased. By contrast, for Georges Bank cod (Fig. 4*g*), R/S has been increasing since roughly 1980, with high recruitment occurring intermittently throughout the time series. Georges Bank yellowtail flounder exhibits yet another pattern (Fig. 4*h*), with R/S relatively constant during 1960–1980, moderately decreasing during the 1980s, and then slightly increasing during the mid-1990s. Recruitment for this stock was high during the 1960s and 1970s and declined substantially after 1980. Georges Bank winter flounder has exhibited an increasing trend in R/S since 1970 (Fig. 4*i*), with a period of relatively high recruitment in the 1980s. Southern New England winter flounder show a cyclical pattern in R/S (Fig. 4*j*), with decreasing trends during the 1960s and late 1970s and increasing trends during the mid-1970s and from the 1980s onwards. Recruitment in this stock appeared to be higher during periods of decreasing R/S. Southern New England yellowtail flounder showed no trend in R/S during 1960–1990 (Fig. 4*k*), but have shown an increasing trend since 1990. Recruitment in this stock was relatively high during the 1960s and during 1980–1981, but has been low otherwise. Overall, the Georges Bank and southern New England stocks exhibited heterogeneous trends in R/S and recruitment.

Changes in R/S values were generally concordant with those predicted by stock–recruitment theory. For the nine stocks that exhibited significant long-term declines in spawning biomass, there was a tendency for R/S values to have increased in recent years. In contrast, the two stocks where spawning stock varied without trend, Gulf of Maine white hake and Gulf of Maine yellowtail flounder, did not have increasing trends in survival ratio in recent years. Thus, the expectation of stock–recruitment theory that R/S should increase as spawning stock decreases was evident across stocks and showed that stock–recruitment models need to reflect this compensatory behavior. In addition, temporal changes in the R/S values of some stocks showed that mod-

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Table 2. Summary of relative rankings of maximum and minimum recruitment (max(R) and (min(R), respectively) ranked by spawner abundance, relative rankings of maximum and minimum recruitment per spawner (max(R/S) and (min(R/S), respectively) ranked by spawner abundance, ratios of expected R/S above median spawner abundance $E[(R/S)_{S>\text{med}(S)}]$ to expected R/S below median spawner abundance $E[(R/S)_{S<\text{med}(S)}]$ and their corresponding one-tailed probability values based on randomization tests for 11 New England groundfish stocks, along with slopes of linear regressions of recruitment versus time and spawning biomass versus time and their two-sided probability values of estimated slopes based on randomization tests.

	Relative ranking of S					Regression slope	
Stock	Max(R)	Min(R)	Max(R/S)	Min(R/S)	$E[(R/S)_{S>\text{med}(S)}]/E[(R/S)_{S<\text{med}(S)}]$	R	S
Gulf of Maine							
Amercian plaice	0.13	0.71	0.13*	0.97**	0.39***	1.34*	–0.19**
Witch flounder	0.23	0.97	0.00***	0.97**	0.09***	2.53***	–2.95***
Cod	0.94*	0.15*	0.12*	0.88*	0.57*	–0.03	–0.74**
White hake	0.28	1.00	0.06*	1.00***	0.48***	0.19*	0.19*
Yellowtail flounder	0.76	0.35	0.06*	0.82*	0.20**	0.14	0.01
Georges Bank							
Haddock	0.95**	0.77	0.09*	0.77*	1.14	–1.36***	–1.57***
Cod	0.64	0.27	0.12*	1.00***	0.50**	–0.44*	–2.63***
Yellowtail flounder	0.67	0.21	0.06*	0.21	0.70*	–2.83***	–0.96***
Winter flounder	0.32	0.06*	0.32	1.00***	0.36***	0.05	–0.19***
Southern New England							
Winter flounder	0.94*	0.13*	0.06*	0.97**	0.62**	–6.80***	–0.87***
Yellowtail flounder	0.73	0.30	0.00***	0.30	0.17**	–2.91***	–1.99***

Note: *, $0.20 \geq P > 0.05$; **, $0.05 \geq P > 0.01$; ***, $P \leq 0.01$.

els with correlated error structures might be needed to account for environmental forcing.

The chances of obtaining high recruitment generally improved when spawning-stock size was high (Fig. 5*a*). Odds ratios were significantly greater than unity for five stocks: Georges Bank haddock and yellowtail and winter flounders and Southern New England yellowtail and winter flounders. Further, the odds ratio was greater than unity for 8 of 11 stocks, the exceptions being Gulf of Maine cod, American plaice, and witch flounder. For two of these three stocks, the odds ratio was less than unity, suggesting the possibility of overcompensatory stock–recruitment dynamics. The recruitment ratio was also above unity for 8 of the 11 stocks (Fig. 5*b*). This implies that there was little risk of reduced recruitment if high spawning stocks were maintained. For many of the 11 stocks, substantial gains in productivity were obvious when S was high. For example, the odds of obtaining high recruitment were over 20 times greater when S was high for the Georges Bank haddock and yellowtail flounder stocks. Conversely, the odds of obtaining high R at low S were less than 1 in 20. Further, the expected year-class size would be over five times greater at high S for these two productive stocks. Overall, the odds ratio analyses indicated that density-dependent factors influence recruitment in most New England groundfish.

Two important patterns emerged from the empirical analyses. First, the stock–recruitment data were highly variable for all stocks. Any model of stock–recruitment dynamics would need to reflect this variability. Second, the 11 stocks exhibited different degrees of density dependence. Hence, no single model could be expected to be suitable for all stocks.

Stock–recruitment dynamics

Selection of a single best model based on AIC_C values led to four models being chosen (Table 3). The constant recruitment with uncorrelated errors model had the minimum AIC_C value for Gulf of Maine cod and yellowtail flounder and Georges Bank cod. The constant recruitment with correlated errors model provided the best fit for southern New England winter and yellowtail flounders. The serially correlated Beverton–Holt model was chosen for three stocks: Gulf of Maine American plaice and Georges Bank haddock and yellowtail flounder. The uncorrelated Ricker model was selected for three stocks: Gulf of Maine witch flounder and white hake and Georges Bank winter flounder. Selected models for some stocks, for example, Gulf of Maine American plaice, Georges Bank haddock, and southern New England winter flounder, had only modest differences in AIC_C, indicating that these selections were not robust.

In contrast, the posterior probabilities explicitly quantified the relative probability of each dynamic model (Table 4), given the available data. For Gulf of Maine American plaice and Georges Bank haddock, there was considerable uncertainty as to the best model, because the odds ratio of the highest posterior probability model to the next most-likely model was roughly one. In contrast, for Gulf of Maine cod, witch flounder, and yellowtail flounder and southern New England yellowtail flounder, there was a high degree of certainty about a stock–recruitment model, with an odds ratio of the most likely model to the next most-likely model of greater than two. There were five other stocks that exhibited a moderate degree of certainty about a stock–recruitment model: Gulf of Maine white hake; Georges Bank cod and yellowtail and winter flounders; and southern New England winter flounder. Odds ratios for these stocks were between roughly 1.4 and 1.8. Thus, stock–recruitment models were well determined for some stocks but relatively uncertain for others.

Eight of the 11 stocks had higher odds of having density-dependent versus density-independent dynamics, based on the posterior model probabilities (Table 4; Fig. 6*a*): Gulf of Maine American plaice, witch flounder, and white hake;

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Fig. 4. Trends in survival ratios (recruitment per spawner), with circle size scaled relative to the magnitude of recruitment by stock, for Gulf of Maine American plaice (*a*), witch flounder (*b*), cod (*c*), white hake (*d*), and yellowtail flounder (*e*); Georges Bank haddock (*f*), cod (*g*), yellowtail flounder (*h*), and winter flounder (*i*); and southern New England winter flounder (*j*) and yellowtail flounder (*k*).

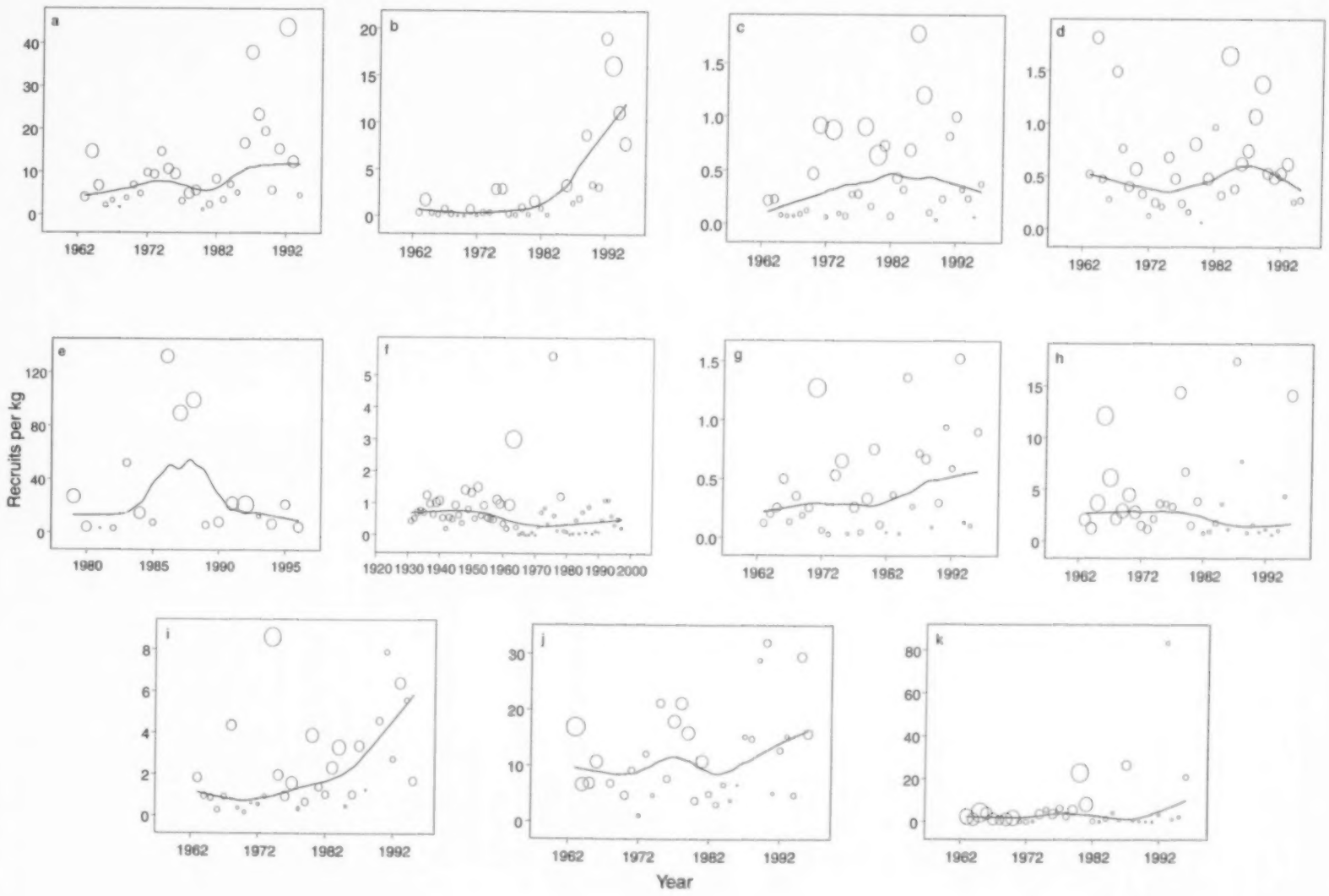

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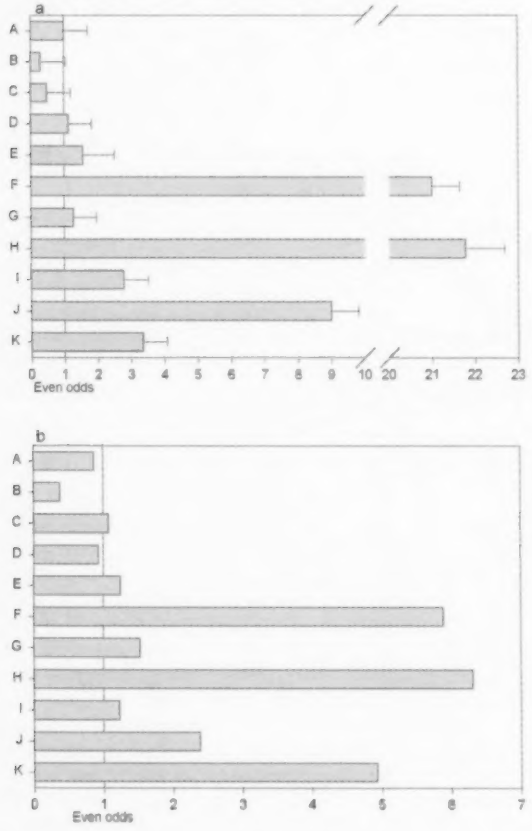
Fig. 5. Odds ratio of obtaining recruitment above the median when spawning-stock size was above its median versus when spawning-stock size was below its median (*a*) and recruitment ratio of geometric mean recruitment above median spawning-stock size to geometric mean recruitment below median spawning-stock size (*b*) for Gulf of Maine American plaice (A), witch flounder (B), cod (C), white hake (D), and yellowtail flounder (E); Georges Bank haddock (F), cod (G), yellowtail flounder (H), and winter flounder (I); and southern New England winter flounder (J) and yellowtail flounder (K).



Georges Bank haddock, cod, and yellowtail and winter flounders; and southern New England winter flounder. Density-independent dynamics were more likely over the range of observed stock–recruitment data for Gulf of Maine cod and yellowtail flounder and southern New England yellowtail flounder (Fig. 6*a*). For most stocks, the stock–recruitment data were relatively informative about whether density-dependent or density-independent dynamics were more likely over the range of observed data.

Similarly, 6 of the 11 stocks had high odds of having uncorrelated versus serially correlated environmental variation, based on the posterior model probabilities (Table 4; Fig. 6*b*). Gulf of Maine witch flounder, cod, white hake, and yellowtail flounder and Georges Bank cod and winter flounder had high odds of having uncorrelated errors. In contrast, four of the remaining five stocks had higher odds of having correlated errors: Gulf of Maine American plaice; Georges Bank haddock and yellowtail flounder; and southern New England yellowtail flounder. For southern New England winter flounder, the odds of serially correlated errors were less than two to one. Thus, for most stocks, it was relatively clear whether uncorrelated or serially correlated environmental variation was more appropriate.

The strength of serially correlated environmental variation differed among stocks (Table 4). Three stocks, Georges Bank haddock and yellowtail flounder and southern New England yellowtail flounder, exhibited strong serial correlation, with values of $E[\phi]$ of roughly ½ or greater. Serial correlation in the stock–recruitment data was moderate for two flounder stocks, Gulf of Maine American plaice and southern New England winter flounder, while serial correlation was negligible for the remaining six stocks. On average, flounders had stronger serial correlation (0.27) than gadids (0.12). Similarly, serial correlation was stronger, on average, for Georges Bank and southern New England stocks (0.34) than for Gulf of Maine stocks (0.07). Thus, taxonomic and geographic differences in serial correlation were apparent.

The expected recruitment at maximum observed spawning biomass per unit area was highest for Georges Bank haddock and lowest for Gulf of Maine white hake. Georges Bank and southern New England stocks had much higher expected recruitments at maximum observed spawning biomass per unit area (Table 4), averaging two and a half times more recruits per area (1281) than Gulf of Maine stocks (508). Similarly, four of the five stocks with expected recruitment at maximum observed spawning biomass per unit area above 1000 were from the continental shelf waters of Georges Bank or southern New England. Thus, geographic differences in potential productivities per unit area were apparent, with Gulf of Maine stocks having the lowest expected values.

There were also consistent differences in expected recruitment at maximum observed spawning biomass per unit area between flounders and gadids (Table 4). Flounders had a higher production of recruits per area at high spawning-stock size, on average (994), than gadids (817). Of the flounders, yellowtail flounder stocks had the highest production of recruits per area at high spawning-stock size, while Gulf of Maine witch flounder had the lowest. Among the gadids, Georges Bank haddock had an order of magnitude greater production of recruits per area at high spawning-stock size than did Georges Bank cod or Gulf of Maine cod or white hake. This suggested that taxonomic and evolutionary traits accounted for some of the differences in recruitment dynamics among stocks.

Discussion

Our results provide strong evidence that spawning-stock size affects recruitment of New England groundfish. Maximum R occurred at relatively high spawner abundance and minimum R occurred at relatively low spawner abundance for the majority of stocks. The stock–recruitment data showed that the maximum survival ratio occurred at low spawner abundance for all stocks, and that survival ratios tended to increase with decreasing spawning-stock sizes. Similarly, the minimum survival ratio occurred at high

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Table 3. Summary of differences (ΔAIC_C) between the bias-corrected Akaike's information criterion (AIC_C) of the best model and the AIC_C values of al ternative stock–recruitment models for 11 New England groundfish stocks.

	ΔAIC_C					
	Constant recruitment		Beverton–Holt		Ricker	
Stock	Uncorrelated errors	Correlated errors	Uncorrelated errors	Correlated errors	Uncorrelated errors	Correlated errors
Gulf of Maine						
Amercian plaice	4.05	0.34	1.57	—	8.04	2.70
Witch flounder	2.05	4.63	2.71	5.79	—	2.72
Cod	—	1.54	2.08	3.76	2.76	4.34
White hake	1.16	3.58	3.39	5.99	—	5.99
Yellowtail flounder	—	2.70	2.82	6.04	8.63	11.90
Georges Bank						
Haddock	36.02	3.36	16.04	—	16.03	0.02
Cod	—	1.72	1.73	2.69	0.95	1.93
Yellowtail flounder	30.89	0.73	5.70	—	5.66	1.12
Winter flounder	1.07	3.24	1.97	4.44	—	2.80
Southern New England						
Winter flounder	4.09	—	1.83	1.78	0.66	1.42
Yellowtail flounder	14.51	—	10.76	1.97	10.79	9.35

Note: The best model is denoted by "—."

Table 4. Posterior probabilities of alternative stock–recruitment models (rounded to the nearest whole percentage), as well as expected values of the autoregressive parameter ($E[\phi]$) and the recruitment (numbers of fish) at maximum observed spawning-stock size per unit area, $E[R(S_{max})]\cdot km^{-2}$, for 11 New England groundfish stocks.

	Posterior probability (%)							
	Constant recruitment		Beverton–Holt		Ricker			
Stock	Uncorrelated errors	Correlated errors	Uncorrelated errors	Correlated errors	Uncorrelated errors	Correlated errors	$E[\phi]$	$E[R(S_{max})]\cdot km^{-2}$
Gulf of Maine								
Amercian plaice	5	31	17	37	1	10	0.32	547
Witch flounder	18	5	13	3	49	13	–0.01	272
Cod	43	20	15	7	11	5	0.05	217
White hake	28	8	9	2	50	2	0.00	96
Yellowtail flounder	64	17	16	3	1	0	0.02	1410
Georges Bank								
Haddock	0	9	0	46	0	45	0.50	2633
Cod	32	14	14	8	20	12	–0.07	323
Yellowtail flounder	0	29	2	42	2	24	0.70	1399
Winter flounder	23	8	15	4	40	10	0.02	239
Southern New England								
Winter flounder	4	32	13	13	23	16	0.25	1190
Yellowtail flounder	0	72	0	27	0	1	0.64	1900

Note: *R*, recruitment; *S*, spawner abundance.

spawning-stock size for 9 of the 11 stocks and the ratio of the expected survival ratio above median spawning stock to that below median spawning stock was less than unity for 10 of the 11 stocks. Thus, for most stocks, spawning stock affected the survival ratios and extreme values of recruitment, as one would expect if compensatory processes were important determinants of recruitment. Furthermore, the odds of higher R at higher S was above unity in 8 of the 11 stocks, showing that higher recruitment was more likely at high spawner abundance and, conversely, that lower recruitment was more likely at low spawner abundance, for most stocks. Of these eight stocks, five showed strong evidence of dependence of recruitment on spawning stock based on the results of the log-linear model: Georges Bank haddock and yellowtail and winter flounders; and southern New England yellowtail and winter flounders. Association between recruitment strength and spawning-stock size in the other stocks was not detected. This is not surprising, given the variability and measurement error in our stock–recruitment data and the short time series in some cases. However, the absence of a statistically significant model does not imply that recruitment is independent of spawning-stock size over

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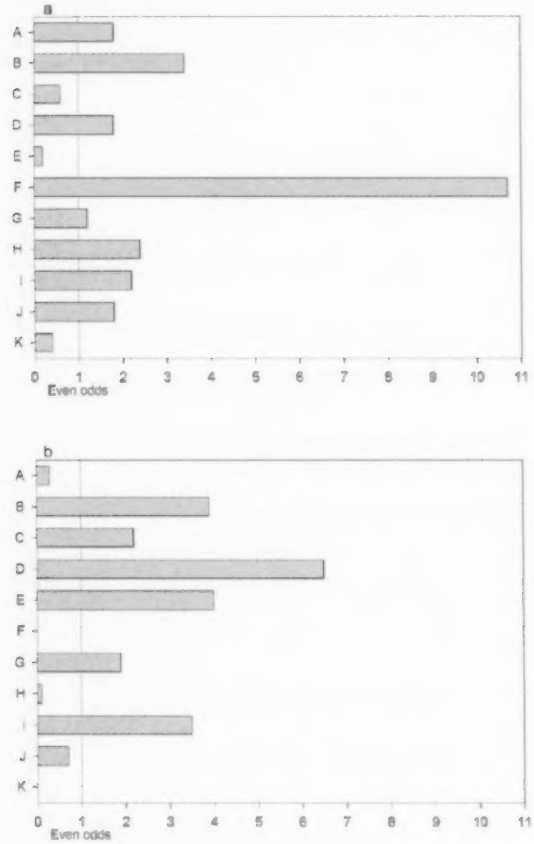
Fig. 6. Odds of density-dependent dynamics versus density-independent dynamics (*a*) and odds of uncorrelated versus serially correlated environmental variation (*b*) for Gulf of Maine American plaice (A), witch flounder (B), cod (C), white hake (D), and yellowtail flounder (E); Georges Bank haddock (F), cod (G), yellowtail flounder (H), and winter flounder (I); and southern New England winter flounder (J) and yellowtail flounder (K).



all ranges of S, and recruitment must eventually decline as spawning stock declines. Although environmental variation undoubtedly influences recruitment strength, in this context the results of Myers and Barrowman (1996) are very important, because they show that, when a large number of stocks (n = 364) is examined, the relationship between spawning stock and recruitment becomes readily apparent.

One important consequence of our results is that the benefit of maintaining higher spawning-stock sizes of New England groundfish is likely to be great. Results suggest that the odds of obtaining higher recruitment are increased when a stock is kept above the median level of spawning biomass. In a majority of cases, odds ratios were greater than one, indicating a better than 50% chance that recruitment would improve if spawning stock was kept above its observed median. Furthermore, recruitment ratios were greater than one for a majority of stocks, indicating that average recruitment was higher when spawning stock was above its median. For some stocks, recruitment can be expected to be markedly higher if spawning stock is increased. For example, when the spawning biomass is kept above the median spawning biomass, the average year-class of haddock is expected to be about six times larger and the odds of above-average recruitment are 21 times greater than when spawning stock is below the median. This would result in larger fishery yields and revenues, more stable stock conditions, and enhanced net benefits (Edwards and Murawski 1993; Overholtz et al. 1995).

The Bayesian approach to depicting uncertainty about stock–recruitment models allows the quantificaton of the relative importance of density dependence and error structure in evaluating alternative stock–recruitment hypotheses. While the use of a suite of stock–recruitment models complicated our analyses, it provided a more robust and accurate depiction of our knowledge of the stock–recruitment dynamics of New England groundfish over the range of observed data, and represented a technical improvement over the assumption that a particular model would be adequate for all stocks based on theoretical grounds (National Research Council 1998). Undoubtedly, further refinements will occur in the understanding of stock–recruitment dynamics of New England groundfish by investigating nonstationarity in stock–recruitment relationships (Sainsbury 1998). For example, recent increases in the abundance of herring and mackerel may have impacted groundfish survival ratios (see, for example, Fogarty and Murawski 1998), and this may be an important trophic interaction to investigate.

The Bayesian analysis also provides the information needed to incorporate stock–recruitment model uncertainty into short-term projections of stock dynamics under alternative rebuilding plans. Given a set of harvest strategies, the probable consequence of applying each strategy can be evaluated by randomly selecting a stock–recruitment model for each simulation of the stock through the rebuilding time horizon. Stock–recruitment dynamics are then simulated according to the deterministic component related to spawning stock and the stochastic lognormal error term that may be correlated or uncorrelated. Simulation results can then be summarized in a decision table (Francis and Shotton 1997) depicting the expected consequences of each harvest strategy under the alternative stock–recruitment models, along with the probabilities that each model represents the true state of nature. While uncertainty in initial population size or other factors may also be incorporated into such projections, explicitly accounting for uncertainty in stock–recruitment dynamics will be a key feature for realistically characterizing the uncertainty in stock rebuilding plans (Brodziak et al. 1998), especially when small differences in goodness-of-fit measures exist between models.

The eight stocks that had better than even odds of having a density-dependent stock–recruitment model (Gulf of Maine American plaice, witch flounder, and white hake; Georges Bank haddock, cod, and yellowtail and winter flounders; and southern New England winter flounder) showed a variety of stock–recruitment dynamics, as indicated by the most likely model. However, model uncertainty was substantial for all of these stocks. The most likely models had probabilities ranging from 32 to 50%, with odds ratios of less than three for the most probable to the next most probable stock–recruitment

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hypothesis. Thus, while most New England groundfish show evidence that recruitment is dependent on spawner abundance, the functional form of this density dependence is uncertain.

In contrast, environmental variation may be an important determinant of recruitment in the three New England groundfish stocks that had less than even odds of having a density-dependent model (Gulf of Maine cod and yellowtail flounder and southern New England yellowtail flounder). Of these, the best model was relatively certain for Gulf of Maine yellowtail flounder (64%) and southern New England yellowtail flounder (72%). Nonetheless, dependence of survival ratio on spawner abundance was generally indicated in these three stocks, as survival ratios declined when spawner abundance was high and minimum and maximum values occurred at high and low spawner abundances, respectively. Although constant-recruitment models may be useful for simulating recruitment in these stocks, it is risky to extrapolate these relationships below the observed range of spawning stock, given the long-term declines in spawning stock for Gulf of Maine cod and yellowtail flounder and Georges Bank cod.

While all stocks exhibited density dependence in survival ratio, only five had better than even odds of having serially correlated environmental variation: Gulf of Maine American plaice; Georges Bank haddock and yellowtail flounder; and southern New England winter and yellowtail flounders. Of these, Georges Bank haddock and yellowtail flounder and southern New England yellowtail flounder had very high probabilities (>95%) of having a stock–recruitment model with autoregressive errors. Although recruitment overfishing is generally believed to be the primary cause of New England groundfish depletion (Sinclair and Murawski 1997), environmental conditions can affect recruitment strength and will likely influence how rapidly some stocks can be rebuilt. Regardless, longer time series of stock–recruitment data will be needed to accurately measure the relative strengths of environmental-forcing versus density-dependent effects over decadal time scales.

Most New England groundfish did not appear to require serial correlation to model stock–recruitment dynamics. Of the six stocks that had less than even odds of having autoregressive errors (Gulf of Maine cod, witch flounder, white hake, and yellowtail flounder; Georges Bank cod and winter flounder), most were from the Gulf of Maine. Because these six stocks had high probabilities of having independent errors (range 66–87%), it was relatively certain that serial correlation was either unimportant or not detectable for these stocks.

Potential recruitment strengths of New England groundfish stocks appeared to differ by region. The continental shelf waters of southern New England and Georges Bank generally have higher primary productivity than Gulf of Maine waters (O'Reilly et al. 1987). The expectation that this productivity funnels upward through the food chain and leads to greater fish production was consistent with our findings. Recent survival ratios of the two most productive Georges Bank stocks (haddock and yellowtail flounder) and the two most productive southern New England stocks (yellowtail and winter flounders) have increased. The causes of these trends are unknown, but may result from regional similarities in environmental forcing that are reflected in the strength of serial correlation in stock–recruitment models for the Georges Bank and southern New England stocks versus Gulf of Maine stocks.

Although environmental forcing can be expected to affect recruitment (e.g., variation in wind stress or water temperature) on a broad scale, we did not attempt to model recruitment synchrony among New England groundfish stocks. Nonetheless, we did observe that recruitment time series of Georges Bank and southern New England yellowtail flounder were highly correlated. Recruitment strengths of these yellowtail flounder stocks may be linked in some years when larvae are advected from Georges Bank to southern New England in water flows through Nantucket Shoals (Mountain and Schlitz 1987). The fact that these stocks exhibited similar empirical patterns of density dependence and serial correlation suggests that further investigation into the recruitment mechanisms for these stocks is warranted.

The strong density dependence of Georges Bank haddock and yellowtail flounder was consistent with previous analyses of these stocks (Overholtz et al. 1986; Brodziak et al. 1998). The rebuilding of these productive stocks to historic abundance levels will require reduced fishing effort but is expected to generate substantial benefits (Overholtz et al. 1995). Our analyses show that, over the long term, survival ratios of these stocks have varied with little or no trend; this implies that higher average recruitment can be expected when spawner abundance is increased. Our analyses also show that these stocks exhibit serial correlation in recruitment dynamics. This is an important feature to model in forecasting the rebuilding of these stocks, although the causes of these correlations (e.g., due to environmental effects or other factors) are unknown.

Some believe that it is not necessary to manage groundfish stocks to maintain spawner abundance and that fisheries exhibit "chaotic" unpredictable behavior making quota-based management infeasible (Wilson et al. 1994). While these notions have been challenged (Fogarty 1995; Hilborn and Gunderson 1996), the pragmatic issue of how best to predict the consequences of alternative management measures remains. Quantitative prediction is model dependent, and different modeling approaches can yield different outcomes. Clearly, our results show that stochastic models are necessary to describe the stock–recruitment dynamics of New England groundfish. While both randomness and chaos imply a lack of system predictability in the absence of perfect information on system state, it is clear that the use of stochastic models to describe recruitment fluctuations is both parsimonious and consistent with the available data. Further, our approach to quantifying the uncertainty in stock–recruitment dynamics provides an objective basis for evaluating the impacts of future harvests on rebuilding plans for depleted stocks.

Three key implications of our findings for rebuilding New England groundfish stocks are: (1) time horizons for stock rebuilding will be uncertain, owing to recruitment variability, (2) some productive stocks (haddock, yellowtail flounder) have serial correlation in recruitment and this may either enhance or diminish chances for rapid stock recovery,

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(3) effective management of New England groundfish will require information on stock dynamics at higher spawning-stock sizes and this, in turn, will require adaptive harvest policies that rebuild spawner abundance.

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Redside shiner (*Richardsonius balteatus*) shoals provide a behavioral competitive refuge for subordinate juvenile steelhead trout (*Oncorhynchus mykiss*)

Craig A. Tinus and Gordon H. Reeves

Abstract: We examined the relative effects of zero, three, and nine redside shiner (*Richardsonius balteatus*) on the aggression-related damage among three juvenile steelhead (*Oncorhynchus mykiss*) in aquaria at 15 and 20°C. No steelhead died when in the presence of nine redside shiner. When redside shiner were not present, mortality among the smallest steelhead was 80%. Thus, the survival of smaller juvenile steelhead was significantly enhanced by the presence of redside shiner. A second experiment was conducted in 6800-L stream channels at 15°C with natural substrate. In separate trials, 10 and 7 steelhead were held either alone or with 20 redside shiner. In the absence of redside shiner, fin damage was significantly greater among smaller steelhead. In both experiments, if a redside shiner group was present, the smallest steelhead frequently took refuge within the shiner group, thereby avoiding attack by dominant steelhead. We have termed this phenomenon a behavioural competitive refuge.

Résumé : Nous avons déterminé les effets relatifs de la présence de zéro, trois et neuf Ménés rouges (*Richardsonius balteatus*) sur l'incidence de dommages dus à l'agression chez trois jeunes Truites arc-en-ciel anadromes (*Oncorhynchus mykiss*) gardées ensemble en aquarium à 15 et à 20°C. Il n'y avait pas de mortalité chez la truite en présence de neuf ménés. En l'absence des ménés, la mortalité chez les plus petites truites était de 80%. Ainsi, la survie des petites truites était significativement améliorée par la présence des ménés. Une seconde expérience a été montée dans des canaux artificiels de 6800 L à 15°C sur substrat naturel, dans laquelle 10 ou 7 truites ont été gardées soit seules, soit en présence de 20 ménés. En l'absence des ménés, les dommages aux nageoires des plus petites truites étaient significativement plus importants. Dans les deux expériences, lorsqu'un groupe de ménés était présent, la plus petite truite se réfugiait souvent au milieu du banc de ménés, échappant ainsi à l'attaque de la truite dominante. Nous appelons ce phénomène un refuge comportemental contre la compétition.

[Traduit par la Rédaction]

Introduction

There are two general mechanisms of competition: (1) exploitation, which is mediated indirectly through a differential efficiency of resource utilization, and (2) interference, which is a direct interaction wherein a dominant either prevents a subordinate from obtaining resources or wounds the subordinate while in defense or acquisition of resources (Colwell and Fuentes 1975). If there are strong differences in competitive ability, either between species or within one species, then less-competitive individuals can coexist with competitive dominants only if there is a competitive refuge. Typically, a competitive refuge exists in the form of spatial or trophic resources that are unavailable or unused by dominants. An example of a spatial refuge from competition is a habitat feature that increases visual isolation and, thus, territory density. To the extent that a point in a resource continuum can be identified as critically limiting, it is useful to identify individuals in terms of being either dominant or subordinate and able to persist or not.

In the case of niche inclusion, where the fundamental niche of a specialist is a subset of that of a generalist, the specialist must be a superior competitor for coexistence to occur (Morse 1974). This concept assumes that density-dependant factors are relevant and that some resources are limiting. The niche space unused by the dominant specialist provides a competitive refuge for the subordinate generalist. If the critical niche dimension is a product of certain behavioral traits, so that the generalist has a greater repertoire of behaviours relative to the specialist, then the ability to change behaviour may serve as a competitive refuge.

Where redside shiner (*Richardsonius balteatus*) and steelhead (*Oncorhynchus mykiss*) are sympatric, shiner (a schooling minnow) are typically found in higher densities than steelhead (Reeves 1984; C.A. Tinus, unpublished data). Steelhead are drift specialists (Abbott and Dill 1985) and

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shiner are more general, feeding both on drifting invertebrates in fast water and foraging benthically in slower water (Lindsey and Northcote 1963). Juvenile steelhead are interference competitors, even when food is abundant, and aggressively defend feeding territories (Hutchison and Iwata 1997). The fates of steelhead unable to hold territories are poorly understood, though some evidence suggests strong density-dependent mortality during spring and summer (Frazer 1969; Crisp 1993; Elliott 1994). Typically, streams in the Pacific Northwest have a period during the summer dry season when available habitat is greatly reduced. It is during these periods, when few species are focusing on a similar limiting resource, that competition should be most detectable.

Because intraspecific competition among steelhead can be strong, subordinate fish may find a competitive refuge in alternative behaviours. One such competitive refuge is to disperse into marginal habitat, which may be less productive or more accessible to predators but not contested (Li and Brocksen 1977; Fausch 1984; Metcalfe 1986). These other habitats may also be physically more complex, offering greater refuge from competition through visual isolation. Foraging efficiency may be reduced if food availability remains constant, however the energetic cost of territorial defense may be reduced. This spatial refuge is an option when stream flows are higher, but may not be available during periods of low flow in summer.

Most experimental studies of competition in cold-water stream fishes have described pairwise interactions between species (Matthews 1998). However, in systems where intraspecific interactions are also strong, or where interactions are strongly affected by multiple species or environmental factors, a pairwise-interaction model may be misleading (Fausch 1998). The objective of this study was to test the effect of redside shiner on intraspecific aggression among juvenile steelhead of different competitive abilities at two different temperatures and at two different spatial scales.

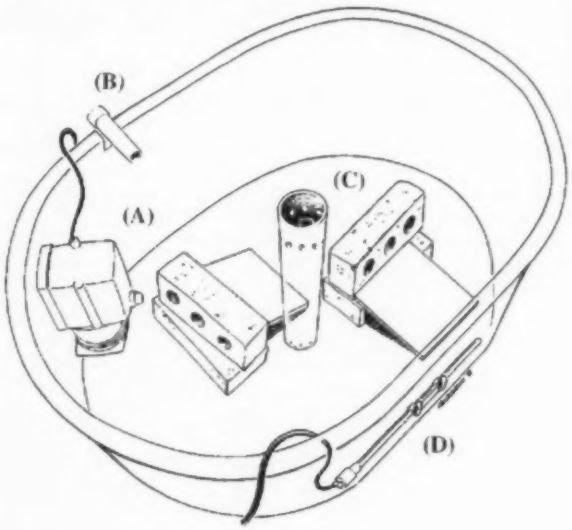
Materials and methods

Fish collection and handling

Sympatric redside shiner and juvenile steelhead were captured by electroshocking in Steamboat Creek in the Umpqua River basin during September and October of 1995 and 1996. Transport time to the laboratory was always less than 5 h and no fish died during transport.

Fish were initially placed in 600-L circular tanks (diameter 0.91 m; depth 0.91 m) in the Oregon Cooperative Fisheries Research Unit hatchery, Oregon State University, Corvallis. Well water at 12.8°C flowed through each tank at 600 L·h^{-1}. Each tank had six 0.3-m sections of 0.2 m diameter sewer pipe cut into half circles and placed on the tank bottom to provide both overhead cover and visual isolation to reduce stress. Steelhead densities ranged from 25 to 50 fish per tank, depending on fish size. Shiner were held at densities of up to 250 fish per tank. Fish were treated against external parasites with malachite green and against bacterial infection with a furan-based antibiotic. Both species were initially fed live 3-week-old crickets, about 30 per tank, and frozen krill (*Euphausia pacifica*), estimated to equal 2.5% of the biomass in each tank, twice daily until all fish were feeding, generally 3–5 days. They were then fed only frozen krill in the same amount as before, twice daily. A photoperiod of 10 h light : 14 h dark was maintained with full spectrum lights under timer control. Fish were held under these conditions for at least 2 weeks before the experimental trials.

Fig. 1. Setup of individual 80-L tanks used in laboratory experiments. A submersible pump (A) maintained a clockwise flow. A slurry of food mixed with water was introduced through a pipe (B) just in front of the pump. Two structures made of two bricks and one tile (C) provided cover. A submersible heater (D) maintained the temperature in trials at 20°C.



Laboratory tank experiment

This experiment was conducted in tanks at the Oregon State Salmon Disease Laboratory in Corvallis, Oreg. A single 25 L·min^{-1} submersible pump within 80-L oval-shaped tanks created water flow. Clean water was added to each tank at 0.5 L·min^{-1}. The primary light source was natural light from overhead windows. Two brick and tile structures in each tank were designed to mimic the "pocket water" areas trout defend as feeding territories in natural streams (Fig. 1).

Fish were fed ad lib, thus reducing exploitative competition and focusing the experiment on the effects of interference competition. There were four different combinations of fish in different tanks at 15 and 20°C: three juvenile steelhead alone, nine shiner alone, three steelhead with three shiner, and three steelhead with nine shiner. There were five replicates of each of the above combinations. Since one juvenile steelhead to three shiner was observed in the stream at the point and time of capture (C.A. Tinus, unpublished data), these ratios were realistic. Each tank was randomly assigned a treatment allocation. Each treatment was rotated in sequential trials among different tanks, providing serial replication. No fish was used more than once. The trial period was 21 days. The order of fish placement was randomized, with the constraint that the sizes of fish in each tank were similar.

Fish were anesthetized in buffered methanotricansulfate (MS222) before being measured to the nearest 1.0 mm, weighed to the nearest 0.1 g, and inspected for signs of disease. Individuals were identified by their parr marks, size, weight, and tank allocation. Individual fish were tracked throughout the experiment. Shiner ranged from 65 to 100 mm in fork length and from 3 to 20 g in wet weight. Steelhead ranged from 90 to 153 mm in fork length and from 8.6 to 41.4 g in wet weight. Because of tissue wasting from disease, physical damage, and osmotic imbalance, dead and dying fish were not used to estimate growth. Pathogens

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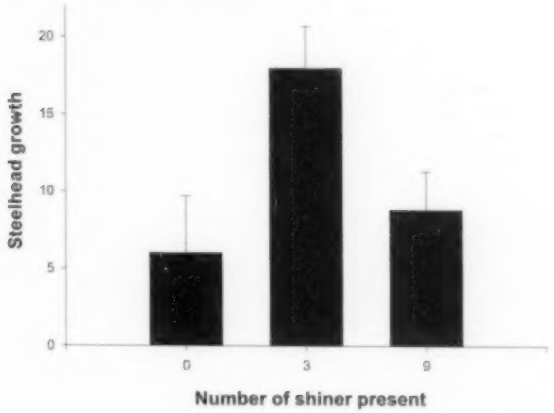
Fig. 2. Mean (+SE) percent change in wet weight (final weight minus starting weight divided by starting weight) of all surviving steelhead tested with zero, three, or nine shiner. Steelhead size classes and temperatures were pooled.



were isolated, identified, and roughly quantified. At the end of the 21-day test period, all surviving fish were removed from the tanks and killed with MS222. Each fish was weighed, measured, identified, and necropsied.

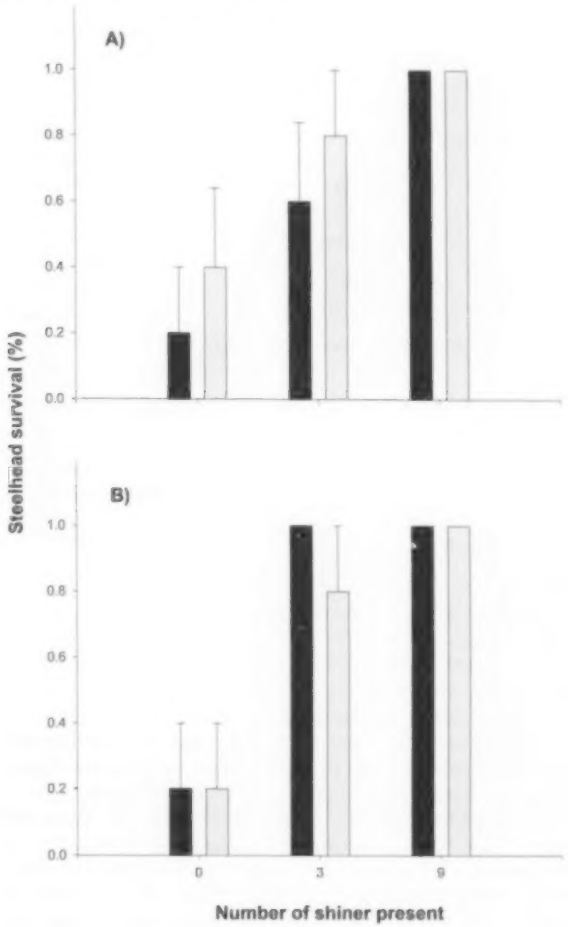
Laboratory stream-channel experiment

This experiment was conducted in two laboratory stream channels at the U.S. Department of Agriculture Forest Service, Pacific Northwest Research Station, Corvallis, Oreg. Each oval channel was 4.3 × 4.9 m on the sides, 0.8 m wide, and 0.61 m high (see Reeves et al. 1983). Channels were divided into two sections of equal size, creating four experimental cells. Inner walls were made of clear Plexiglas; opaque curtains surrounded the channels, limiting outside light and disturbance and allowing an observer to be unnoticed by the fish. Substrate was pea gravel (6–12 mm) in each pool and gravel (20–50 mm) mixed with cobble (100–500 mm) in the riffles. Two areas 35 cm deep and two areas 50 cm deep were created in each section.

Water was circulated through filters and ultraviolet sterilizers. A Plexiglas paddle wheel in each channel maintained water flow at about 0.9 $m \cdot s^{-1}$. Water temperature was maintained at 15°C. The photoperiod was maintained at 15 h light : 9 h dark, with 2 h brightening and 2 h dimming phases included in the light period to simulate twilight. Daily rations (5% of the total wet biomass in each section) of krill were fed in two daily feeding periods, one in midmorning and one in midafternoon. The food was frozen in blocks of ice, allowing particles to be delivered to the system gradually (by thawing) over a 1-h period, to avoid the artifact of fish reacting to a sudden pulse of food.

Fish were taken from holding tanks and randomly assigned to a channel section. The four sections were each randomly assigned a treatment. In the first trial, 20 shiner were added to two sections, with10 steelhead being added 12 h later. Ten steelhead only were then added to the remaining two sections. In a second trial, 20 shiner and seven steelhead were used in the same manner as in the first trial. In this experiment, only the relative presence of steelhead with shiner varied. This arrangement provided two replicates per trial and no fish were used more than once. All fish were weighed and measured as in the first experiment, and parr marks were recorded to identify individual fish. Any fin damage was noted for each fish. Trials ran for 10 days and no fish died during the trials. After 10 days, all fish were removed from the channels, by bubbling CO_2 through the water, and killed with MS222. All

Fig. 3. Mean (+SE) percent survival of intermediate-size steelhead (*a*) and of the smallest steelhead (*b*) in each treatment: solid bars, treatments at 15°C; shaded bars, treatments at 20°C. The largest steelhead all survived.



fish were weighed, measured, and identified. Fin tears, which indicate aggressive interaction between steelhead, were assessed (Moutou et al. 1998). Fin tears of less than 30% of the total fin length were given a score of 0.5 and tears of more than 30% of the total fin length were given a score of 1.0. The total fin damage score for each fish was then recorded.

Results

Laboratory tank experiment

The growth of surviving steelhead, defined as the proportional change in wet weight, was significantly greater in the presence of three shiner than in the presence of either zero or nine shiner (analysis of variance: $F_{2,65} = 4.68$, $P = 0.013$; Fig. 2). Steelhead survival was analyzed by logistic regression with a drop-in-deviance test. The largest steelhead all survived and so they were excluded from the model. The presence of both three and nine shiner had a significant and positive effect on both intermediate-sized steelhead ($\chi^2 = 14.11$, df =1, $P < 0.005$) and the smallest steelhead ($\chi^2 =$

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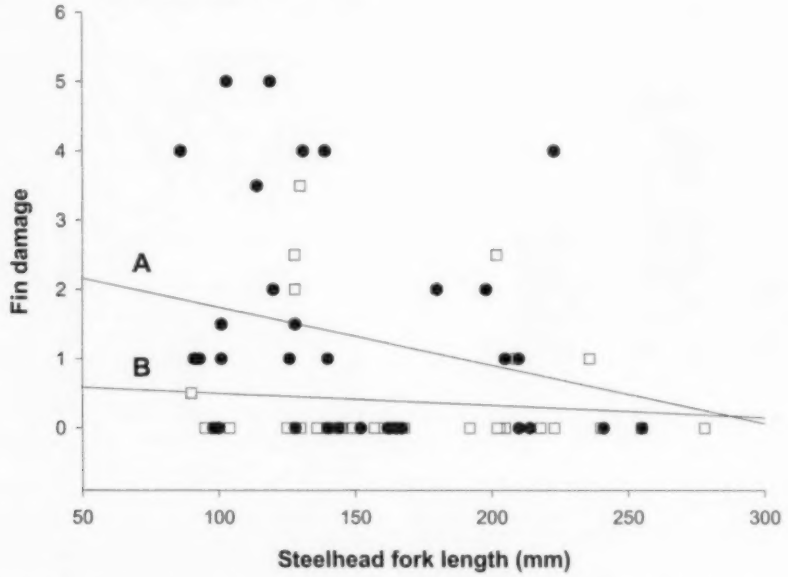
Fig. 4. Linear regression of fin damage with steelhead size. A fin tear of less than 30% of the total fin length was scored as 0.5 and of more than 30% as 1.0. A total score was then recorded for each fish. The regression lines are for steelhead without shiner (solid circles (A)) and steelhead with shiner (open squares (B)).



18.1, df = 1, $P < 0.005$; Fig. 3). There were five replicates. A replicate was a unique combination of fish at one temperature. Temperature was not a significant factor in this experiment. These findings support the hypothesis that shiner reduce the effects of intraspecific aggression in steelhead while also competing for food.

Shiner survival was also analyzed with a logistic-regression model. There were no treatments with three shiner alone, so the effects of shiner density and the presence of steelhead were confounded. However, in treatments where three shiner were with three steelhead, shiner survival was significantly lower than in treatments where nine shiner were either alone or with steelhead ($\chi^2 = 14.11$, df = 1, $P < 0.005$). This result suggests that shiner benefited from higher density in the presence of steelhead. Shiner growth was significantly lower in the presence of steelhead (analysis of variance, ANOVA: $F_{[1,178]} = 60.05$, $P < 0.001$. Again, temperature was not a significant factor.

Laboratory stream-channel experiment

Fin damage, analyzed by a Poisson regression with a drop-in-deviance test, was significantly higher among smaller steelhead when no shiner were present ($\chi^2 = 10.7$, df = 3, $P < 0.005$; Fig. 4). The presence of shiner did not affect steelhead growth in the channel experiment.

Discussion

The laboratory tank experiment showed that the presence of a group of shiner significantly improved the survival of subordinate steelhead and that different temperatures did not affect this result. The experiment in the stream channels more closely approximated natural fish densities and environmental conditions. This experiment suggested that subordinate steelhead were able to escape attack from dominant steelhead by taking refuge in redside shiner shoals. Small steelhead suffered significantly more fin damage when a shiner group was not present.

Redside shiner are known to compete exploitatively for food with juvenile salmonids in both lotic and lentic systems (Lindsey and Northcote 1963; Miller 1967). Shiner have been shown to be more efficient food finders than salmonids under some conditions (Crossman and Larkin 1959). Previous work has shown that temperature is important in determining the outcome of shiner–steelhead competitive interactions in streams (Reeves et al. 1987). In cool water, steelhead dominate areas rich in drifting food by threat displays, chasing, and nipping intruders (Hartman 1965). However, shiner are metabolically more efficient at higher stream temperatures and can switch between feeding benthically in uncontested areas of slow water and making forays into steelhead territories (Reeves et al. 1987). This may allow shiner to persist in a wide range of stream habitats. That temperature was not a significant factor in these experiments was surprising. At the higher temperature, steelhead were expected to be temperature stressed. However, competition effects seem to have been much stronger than any measurable effect between the two temperatures tested. Temperature might have been significant had a wider range of temperatures been tested. We chose 15 and 20°C, because these tempertures were similar to those in streams in which we were working, and because it is at these temperatures that we expected competition to be most pronounced. Reeves et al. (1987) examined both physical and biological factors, but treated steelhead as a monotypic group, and possible differences in the interactions between subordinate steelhead and shiner were not examined.

Fin damage in the stream-channel experiment indicated that smaller steelhead received injury in aggressive interactions and would likely have died from the effects of stress

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and disease if the experiments had run longer (Holt et al. 1975; Schreck 1992). In these experiments, dominance was inferred from fish size. We considered the smallest steelhead to be subordinate in a dominance hierarchy, although some workers have suggested that other factors such as "fierceness," and not strictly size, determine social standing (Huntingford et al. 1990; Titus and Mosegaard 1991; Keeley and McPhail 1998). Even if size was not the correct indicator of social position, the experiments showed that more steelhead were able to live within a given area in the presence of an equal or greater number of shiner than were able to live without shiner.

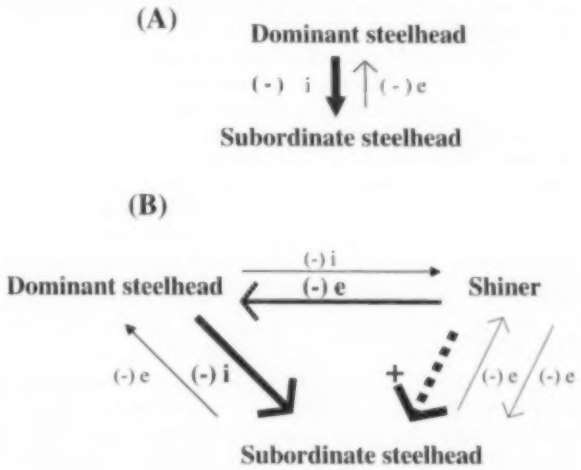
Agonistic behavior between juvenile steelhead may lead to increased mortality of subordinate individuals (Abbott and Dill 1985). Subordinate juvenile steelhead must (*i*) leave an area that is known to contain food and other resources but is also occupied by dominant steelhead, (*ii*) remain and continue to challenge more dominant individuals for food, or (*iii*) modify their behaviour so they can remain in the area and not suffer from constant attacks. One way of accomplishing the third option is to take advantage of the feeding behavior of another group of fish that successfully feed in the desirable area occupied by dominant steelhead.

In this study, the redside shiner regularly moved into areas where drifting food was abundant, bringing them into direct competition with steelhead occupying feeding territories. One explanation for the observed pattern is that, when only steelhead were present, subordinates continually moved into these focal points and suffered physical damage from attack. When shiner were present, the subordinate steelhead moved into the focal points within the shiner group. There may have been a "swarm effect" when a dominant's territory was invaded simultaneously by many intruders, as also occurs among coral-reef fishes (Robertson et al. 1976). There may also have been a "dilution effect," in which a fixed number of attacks are distributed among a greater number of fish. To separate the effects of shiner presence from the effects of increased density, an experimental treatment that maintained fish density would be required. We did not do this, because we wanted to test steelhead at densities similar to those found in natural pools.

Associating closely with a group of shiner may represent a balance of costs and benefits. The cost to subordinate steelhead from association with a group of shiner would be additional exploitative competition for food. The benefit is a reduction in stress and physical damage from interference competition, which might result if subordinates continually challenged dominants. Field observations suggest that shiner respond to stress by increasing density, that is, by reducing nearest-neighbor distances. Shiner may compete more effectively when in groups, although this has not been explicitly tested. In these experiments, when shiner and steelhead were at equal densities (three and three), the redside shiner suffered significantly higher mortality and lower growth.

Figure 5*a* presents a model describing a possible mechanism for the patterns observed in these experiments. In this model, dominant steelhead have a strong negative effect on subordinant steelhead from the standpoint of both exploitation and interference competition. There is a much weaker negative effect on dominant steelhead from the cost of territorial defense. Figure 5*b* illustrates how shiner may have a moderate negative effect on dominant steelhead by defeating their ability to monopolize resources. There is a much weaker negative effect on shiner from the energetic cost of competing with dominant steelhead. When shiner are present, there may be a strong positive indirect effect of shiner on subordinant steelhead. Additionally, in this model, there are weak negative direct effects. These effects represent the possible cost of exploitation competition, when steelhead and shiner shoal together.

Fig. 5. Dominant juvenile steelhead may have a strong negative effect on subordinate steelhead in the absence of other competitors for food. In these descriptive models, the relative strength of the interaction effect is indicated by the width of the arrow, *i* indicates interference competition, *e* indicates exploitation competition, and the sign indicates whether the interaction is positive or negative in the direction of the arrow. (A) Competitive interactions between dominant and subordinate steelhead. (B) Redside shiner moderate interactions between dominant and subordinate steelhead. In this model, the negative direct effect of shiner shoals on territorial steelhead produces an indirect positive effect on subordinate steelhead (dashed arrow).



Under some circumstances, competitively subordinate steelhead require a refuge from dominant interference competitors in order to persist. In this case, individual steelhead that cannot successfully hold territories may shift their behaviour to take advantage of the competitively successful shoaling behaviour of redside shiner. Subordinate steelhead may use redside shiner shoals as a refuge, providing there is an area of overlap in resource use between steelhead and shiner.

The results of these experiments may be relevant to natural populations for several reasons. First, the pattern of shiner effect on steelhead was similar over a broad range of fish densities (from 0.0375 fish/L to 0.0024 fish/L). Second, field observations corroborate the pattern of juvenile steelhead feeding on the fringes of redside shiner groups, while other juvenile steelhead hold feeding territories nearby (C.A. Tinus, personal observation; G.H. Reeves, unpublished data). Third, the summer dry season produces isolated pool habitats in streams where redside shiner and steelhead are sympatric. The natural habitat available to these fishes is a patchwork of both soft-edged and hard-edged habitat types

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(sensu Stamps et al. 1987). Experiments in aquaria are typically hard-edged, meaning a monotypic habitat with impassable boundaries. Stream channels are much less so, because the habitat may be a combination of high-quality and marginal habitat in terms of structure, food availability, and hydrology. A strong pattern demonstrated in both aquaria and stream channels could reasonably be expected to exist in naturally isolated pools that are often relatively homogeneous and may have impassable boundaries. Patterns observed in aquaria are likely to be less pronounced in nature. However, these patterns may still exist in nature and have meaningful effects over longer time scales. Future research will include field manipulation of fish density in naturally isolated habitats.

The long-term effects of subordinate steelhead associating with shiner are unknown. Whether subordinate steelhead that change their behaviour to better compete with dominants have greater fitness or even survive to smolt is unknown. However, these experiments suggest that redside shiner may allow juvenile steelhead to exist at greater densities by providing a refuge from interference competition. If there is a period when density-dependent mortality is being contributed to by interference competition, then behavioural refuges may reduce the per capita mortality rate.

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Floodplain rearing of juvenile chinook salmon: evidence of enhanced growth and survival

T.R. Sommer, M.L. Nobriga, W.C. Harrell, W. Batham, and W.J. Kimmerer

Abstract: In this study, we provide evidence that the Yolo Bypass, the primary floodplain of the lower Sacramento River (California, U.S.A.), provides better rearing and migration habitat for juvenile chinook salmon (*Oncorhynchus tshawytscha*) than adjacent river channels. During 1998 and 1999, salmon increased in size substantially faster in the seasonally inundated agricultural floodplain than in the river, suggesting better growth rates. Similarly, coded-wire-tagged juveniles released in the floodplain were significantly larger at recapture and had higher apparent growth rates than those concurrently released in the river. Improved growth rates in the floodplain were in part a result of significantly higher prey consumption, reflecting greater availability of drift invertebrates. Bioenergetic modeling suggested that feeding success was greater in the floodplain than in the river, despite increased metabolic costs of rearing in the significantly warmer floodplain. Survival indices for coded-wire-tagged groups were somewhat higher for those released in the floodplain than for those released in the river, but the differences were not statistically significant. Growth, survival, feeding success, and prey availability were higher in 1998 than in 1999, a year in which flow was more moderate, indicating that hydrology affects the quality of floodplain rearing habitat. These findings support the predictions of the flood pulse concept and provide new insight into the importance of the floodplain for salmon.

Résumé : Notre étude démontre que le canal de dérivation Yolo, la principale plaine d'inondation de la région aval de la rivière Sacramento (Californie, É.-U.), offre de meilleurs habitats pour l'alevinage et la migration des jeunes Saumons Quinnat (*Onchorhynchus tshawytscha*) que les bras adjacents de la rivière. En 1998 et 1999, la taille des saumons a augmenté plus rapidement dans la plaine d'inondation agricole, sujette aux débordements saisonniers de crue, que dans la rivière, ce qui laisse croire à de meilleurs taux de croissance. De plus, des jeunes saumons marqués à l'aide de fils de métal codés et relâchés dans la plaine d'inondation étaient plus gros au moment de leur recapture et avaient des taux de croissance apparente plus élevés que des poissons relâchés dans la rivière en même temps. L'amélioration des taux de croissance dans la plaine de débordement résultait en partie d'une consommation significativement plus importante de proies, le reflet d'une plus grande disponibilité des invertébrés de la dérive. Un modèle bioénergétique laisse croire que le succès de l'alimentation a été meilleur dans la plaine d'inondation que dans la rivière, en dépit du coût métabolique d'alevinage significativement plus grand dans les eaux plus chaudes de la plaine d'inondation. Les indices de survie des poissons marqués et relâchés dans la plaine d'inondation étaient quelque peu plus élevés que ceux des poissons de la rivière, mais les différences n'étaient pas statistiquement significatives. La croissance, la survie, le succès de l'alimentation et la disponibilité des proies étaient tous supérieurs en 1998 par comparaison avec 1999, une année à débit plus modéré, ce qui indique que l'hydrologie affecte la qualité des habitats d'alevinage dans la plaine d'inondation. Nos résultats appuient les prédictions du concept de pulsion de crue (flood pulse concept) et mettent en lumière l'importance de la plaine d'inondation pour le saumon.

[Traduit par la Rédaction]

Introduction

Although the trophic structure of large rivers is frequently dominated by upstream processes (Vannote et al. 1980), there is increasing recognition that floodplains plays a major role in the productivity and diversity of riverine communities (Bayley 1995). Based largely on observations from relatively undisturbed river–floodplain systems, Junk et al. (1989) proposed the flood pulse concept, which predicts that annual inundation is the principal force determining productivity and biotic interactions in river–floodplain systems. Floodplains can provide higher biotic diversity (Junk et al. 1989) and increased production of fish (Bayley 1991; Halyk and Balon 1983) and invertebrates (Gladden and Smock 1990). Potential mechanisms for floodplain effects include increased habitat diversity and area (Junk et al. 1989), large inputs of

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terrestrial material into the aquatic food web (Winemiller and Jepsen 1998), and decreased predation or competition due to intermediate levels of disturbance (Corti et al. 1997). Nonetheless, the degree to which floodplains support riverine ecosystems remains poorly understood, particularly in regulated and temperate rivers. Uncertainties about river–floodplain relationships are due, in large part, to the difficulty in separating the relative contribution of floodplain versus channel processes and sampling problems in seasonal habitats, which are frequently subject to extreme environmental variation.

In the this study, we examined the relative importance of floodplain and riverine habitat to juvenile chinook salmon (*Oncorhynchus tshawytscha*) in the Sacramento River (California, U.S.A.), a large regulated river (Fig. 1). The system is particularly well suited to a comparative study, because young salmon migrating down the lower Sacramento River to the San Francisco Estuary in wet years have two alternative paths: they may continue down the heavily channelized main river or they may pass through the Yolo Bypass, an agricultural floodplain bordered by levees. We had two reasons to believe that the floodplain might be important habitat for young salmon. First, years of high flow are known to enhance populations of a variety of species in the San Francisco Estuary (Jassby et al. 1995) and the survival of chinook salmon (Kjelson et al. 1982). However, the specific mechanisms for these benefits have not been established. Possible reasons for the positive effects of flow on fish include increased habitat availability, migration cues, food supply, larval transport, and reduced predation rates (Bennett and Moyle 1996). Floodplain inundation is one of the unique characteristics of wet years, during which the Yolo Bypass is likely to be a significant migration corridor for young chinook salmon in the Sacramento Valley. During high-flow events, the Yolo Bypass can convey >75% of the total flow from the Sacramento River basin, the major producer of salmon among tributaries of the San Francisco Estuary. Second, floodplains are known to be among the most important fish-rearing areas in a variety of river systems, yet in developed regions, the availability of this habitat has been greatly reduced by channelization and levee and dam construction (Rasmussen 1996). A high degree of habitat loss may greatly enhance the biological significance of remnant floodplains in heavily modified systems, such as the San Francisco Estuary and its tributaries.

This study tests the hypothesis that the agricultural floodplain provides better habitat quality than the adjacent river channel. For the purpose of this analysis, we focus on salmon growth, feeding success, and survival as indicators of habitat quality. Obviously, there are many other possible measures of habitat quality, such as reproductive output of adults or physiological indicators. However, we believe that the chosen suite of parameters is reasonably representative of habitat quality. For example, Gutreuter et al. (2000) successfully used growth as a factor to test the hypothesis that floodplain inundation had a major effect on fish production.

The San Francisco Estuary is one of the largest estuaries on the Pacific Coast (Fig. 1). The system includes downstream bays (San Pablo and San Francisco) and a delta, a broad network of tidally influenced channels that receive inflow from the Sacramento and San Joaquin rivers. The estuary and its tributaries have been heavily altered by levees, dams, land reclamation activities, and water diversions. The primary floodplain of the Sacramento River portion of the delta is the Yolo Bypass, a 24 000-ha leveed basin that conveys excess flow from the Sacramento Valley, including the Sacramento River, Feather River, American River, Sutter Bypass, and westside streams. The 61 km long floodplain floods seasonally in winter and spring in about 60% of years, and is designed to convey up to 14 000 $m^3 \cdot s^{-1}$. During a typical flooding event, water spills into the Yolo Bypass via the Fremont Weir when Sacramento Basin flows surpass approximately 2000 $m^3 \cdot s^{-1}$. Except during extremely high flow events, the mean depth of the floodplain is generally less than 2 m, creating broad shoal areas. During dry seasons, the Toe Drain channel, a permanent riparian corridor, remains inundated as a result of tidal action. At higher levels of Sacramento Basin flow (e.g., >5000 $m^3 \cdot s^{-1}$), the Sacramento Weir is also frequently operated. Agricultural fields are the dominant habitat type in Yolo Bypass, but approximately one-third of the floodplain area is natural vegetation, including riparian habitat, upland habitat, emergent marsh, and permanent ponds.

There are four races of chinook salmon in the Sacramento Valley: winter, spring, late fall, and fall run (Yoshiyama et al. 2000). Historical data indicate that all races have de-

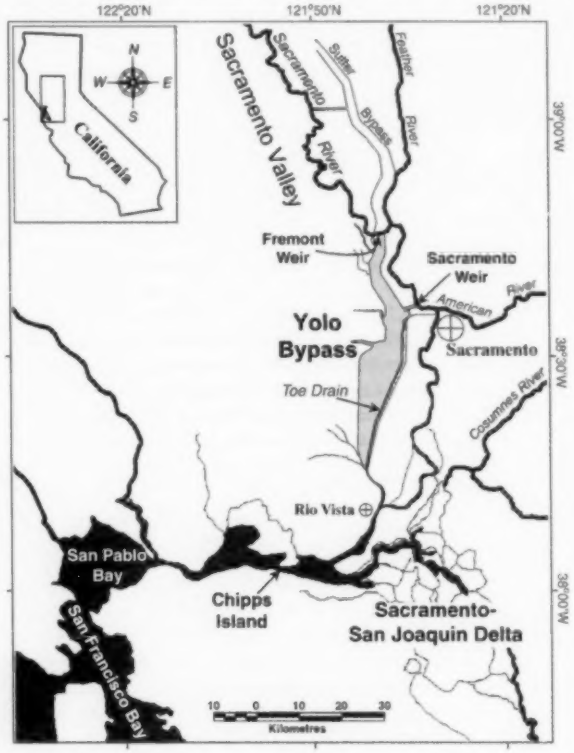

Fig. 1. The location of Yolo Bypass in relation to the San Francisco Estuary and its tributaries. The San Francisco Estuary encompasses the region from San Francisco Bay upstream to Sacramento. Feather River Fish Hatchery is located on the Feather River approximately 112 km upstream of Yolo Bypass.

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creased in abundance since the 1950s, but the spring, winter, and late-fall runs have shown the most pronounced declines. There are multiple causes for these long-term reductions, including habitat loss, habitat degradation, water diversions, and oceanic conditions. In the present study, we focused on the fall run, the numerically dominant race in the Sacramento Valley. The typical life-history pattern for these salmon is for young to migrate from the tributaries to the bay–delta area at the "fry" stage (Brandes and McLain 2001), when most individuals are approximately 35- to 70-mm fork length (FL). In low flow years, there may be substantial upstream rearing in the Sacramento River. Peak juvenile emigration from the tributaries occurs during winter and spring (Kjelson et al. 1982).

Materials and methods

Physical conditions

During 1998–1999, flow measurements in Yolo Bypass and the adjacent stretch of the Sacramento River were obtained from gauges operated by the U.S. Geological Survey (USGS). Daily water temperatures for each site were calculated as the mean of maximum and minimum daily measurements for single stations in the Sacramento River (USGS) and a temperature recorder (Onset Corp.) installed in the Yolo Bypass Toe Drain channel (Fig. 1). However, from 1 February to 26 March 1998, these data were not available for Yolo Bypass. During this period, before the recorder was installed, discrete measurements were taken at the same location, typically during mid or late morning.

Fish sampling

Salmon FL (mm) was measured during January–April in 1998 and 1999 on samples collected with 15-m beach seines (4.75-mm mesh). Samples were collected weekly at five core locations located around the perimeter of the Yolo Bypass, during periods when the basin was flooded. After the bypass drained, additional samples were collected at random locations around the perimeter of ponds near the core locations. Comparative data on salmon size in the adjacent reach of the Sacramento River were collected by the U.S. Fish and Wildlife Service (USFWS) at five beach-seine sites, using techniques similar to those used when the the bypass was flooded.

FLs of salmon obtained from beach-seine sampling were compared to determine whether there was evidence of major differences in salmon size between the Yolo Bypass and the Sacramento River. However, these data were not considered unambiguous evidence of growth differences, because the two systems were open to immigration and emigration during much of the study, and migrating salmon include multiple races of salmon that cannot be readily separated. We addressed this issue by using paired releases of coded-wire-tagged (CWT) juvenile salmon in Yolo Bypass and the Sacramento River. This approach allowed comparisons of growth among fish of similar origin and provided a relative estimate of migration time and survival. The salmon were produced and tagged at the Feather River Fish Hatchery and released on 2 March 1998 and 11 February 1999. The release sites were in Yolo Bypass below Fremont Weir (52 000 in 1998; 105 000 in 1999) and in the adjacent reach of the Sacramento River (53 000 in 1998; 105 000 in 1999). The fish had a mean FL of 57.5 ± 0.5 mm (SE) in 1998 and of 56.8 ± 0.4 mm (SE) in 1999. A small portion of each group was subsequently collected by trawling at the seaward margin of the delta at Chipps Island, which is located downstream of the confluence of the Yolo Bypass and the Sacramento River (Fig. 1). The USFWS Chipps Island survey samples a single channel location with a midwater trawl towed at the surface (Baker et al. 1995; Brandes and McLain 2001). Ten 20-min tows were made each day, except during March in 1998 and 1999, when sampling was conducted every other day. Data on migration time (days) and FL (mm) were recorded for fish recaptured from each release group. Apparent growth rate was also calculated for each fish, as: (FL of individual at Chipps Island – mean FL of CWT release group) × (migration time)$^{-1}$. Survival indices of the paired CWT releases were calculated by USFWS by dividing the number of fish recovered for each release group at Chipps Island by the number released, corrected for the fraction of time and channel width sampled (Brandes and McLain 2001).

Diet

We performed diet comparisons on fall-run juvenile salmon (33–81 mm) collected in beach-seine samples during February–March of 1998 and 1999 from the Yolo Bypass (103 individuals) and the Sacramento River (109 individuals). Fish samples were tagged and stored individually in a deep freeze. After thawing, stomachs were removed from the fish and the contents were identified (using a dissecting microscope) to order (insects and arachnids), genus (crustaceans), or phylum (rarely eaten taxa such as oligochaetes). To develop average invertebrate length estimates, up to 10 individuals of each prey type encountered were measured. Prey dry weight estimates were calculated from average lengths, using regression equations for delta crustaceans obtained from J. Orsi (California Department of Fish and Game, Stockton, CA 95205, unpublished data) and from literature sources. Diet results were compared as an index of relative importance (IRI) (Shreffler et al. 1992) for each month. The index was calculated as: IRI = (% numeric composition + % weight composition) × % frequency of occurrence.

Prey availability

Invertebrates were sampled in February–March of 1998 and 1999, to examine prey availability in the Yolo Bypass and the Sacramento River. Sampling was not designed as a comprehensive evaluation of spatial and temporal variation of prey. Rather, it was intended to provide information on whether variation in salmon diets between the two locations was consistent with gross differences in prey type or relative abundance. We focused on Diptera (adults, pupae, and larvae) and crustacean zooplankton, which comprised over 90% of the diets of Yolo Bypass and Sacramento River juvenile salmon. Weekly drift samples were collected at fixed stations on the Yolo Bypass and the Sacramento River during periods when the floodplain was inundated. The sampling points were located away from overhanging vegetation and bank eddies, in water velocities of approximately 15–60 cm·s^{-1}, depending on flow. Net (500-µm mesh) dimensions were 0.46 × 0.3 m mouth and 0.91 m length. The nets were fished for approximately 30 min during midmorning, to coincide with the time period when most fish-stomach samples were taken. Sample volume was calculated using a flowmeter (General Oceanics Model 2030R) and net dimensions. Drift samples were stored in ethanol or formaldehyde, then identified to family or order using a dissecting microscope. In 1998, zooplankton were collected in the Yolo Bypass at two fixed stations with battery-operated rotary-vane pumps with a mean flow rate of 17 L·min^{-1}. Samples were taken via pipes with outlets at multiple locations beneath the water surface. Discharge was directed into a 150 µm mesh net held in a basin on the bank. Flow rate was recorded at the beginning and end of the sample period, which varied from 1 to 6 h. No samples were taken in the Sacramento River during a comparable period in 1998. In 1999, zooplankton samples were taken with a Clarke–Bumpus net (160-µm mesh, diameter 0.13 m, length 0.76 m) placed in surface flow in the Yolo Bypass and Sacramento River. Sample volume was recorded as for the drift net. Zooplankton samples were concentrated and stored in 5%

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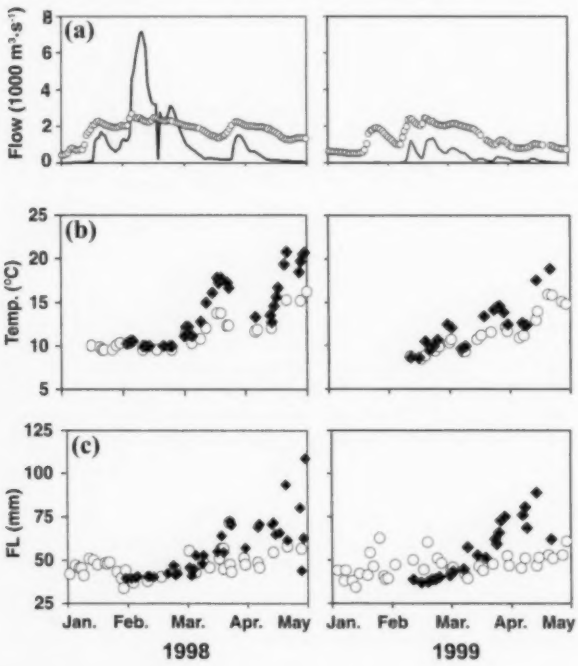
Fig. 2. Chinook salmon size versus physical conditions in Yolo Bypass and the Sacramento River during winter and spring in 1998 and 1999. (*a*) Mean daily flow ($m^3 \cdot s^{-1}$) in Yolo Bypass (solid line) and the Sacramento River (circles). (*b*) Mean water temperature (°C) in Yolo Bypass (solid symbols) and the Sacramento River (open symbols). (*c*) Mean daily chinook salmon FL for Yolo Bypass (solid symbols) and Sacramento River (open symbols) beach-seine stations. For presentation purposes, only the daily mean FLs are shown; however, individual observations for February–March were used for statistical analyses.



formaldehyde, for later identification to genus using a dissecting microscope.

Bioenergetics

Feeding success was examined in two ways: (1) prey biomass estimated from stomach contents and (2) prey biomass estimated as a function of maximum theoretical consumption. For the first measure, we used the previously described stomach-content data to calculate total-prey biomass for individual fish.

A limitation of using prey biomass as a measure of feeding success between locations is that thermal history affects how consumption alters growth rate (Hewett and Kraft 1993). As will be discussed in further detail, water temperatures were significantly higher in the Yolo Bypass floodplain than in the Sacramento River. To correct for this problem, our second approach used bioenergetic modeling to incorporate the metabolic effects of water temperature. We used methods similar to those of Rand and Stewart (1998) to calculate a wet weight ration index, which uses prey biomass for each sampled individual as a proportion of the theoretical maximum daily consumption. The stomach-content data were used as our estimate of prey biomass for individual fish. The theoretical maximum daily consumption rate (C_{max}) was modeled using Fish Bioenergetics 3.0 (Hanson et al. 1997), using observed body size and water temperature at the time each beach-seine sample was collected. The model input also required fish mass, which we estimated from FL data, using length–weight relationships from Sacramento River juvenile salmon (Petrusso 1998). The caloric value of the prey was taken from weight conversion factors provided by Hanson et al. (1997). Model parameters were derived from those of Stewart and Ibarra (1991) for chinook salmon. The model was run for individual fish collected at each sampling location in 1998 and 1999.

We emphasize that the second approach provides an *index*, rather than an *absolute* measure of feeding success. The wet weight ration index is conceptually analogous to "*P*" in Hanson et al. (1997), a model parameter that indicates what fraction of C_{max} is obtained over the course of the day. The major difference is that *P* is based on prey consumption over a 24-hour period, whereas our wet weight ration index is based on instantaneous measurements of stomach contents, which may not represent mean trends over the entire day. An additional limitation is that the Stewart and Ibarra (1991) model parameters were developed for adult salmon and we applied the model to juveniles. We did not have sufficient field or laboratory data to develop bioenergetic-model parameters specific to the earliest life stages. Nonetheless, other studies (Rand and Stewart 1998) have demonstrated that similar wet weight ration indices can provide an effective technique for comparing relative salmonid feeding success between seasons and years.

Table 1. Robust regression statistics for Yolo Bypass and Sacramento River salmon FLs for 1998 and 1999.

	1998		1999	
	Parameter ± SEM	*t*	Parameter ± SEM	*t*
Intercept	29.4±0.6	46.8	23.5±0.5	43.7
Location	6.4±0.6	10.2	11.1±0.5	20.6
Day	0.3±0.01	34.5	0.3±0.01	48.5
Location:day	−0.14±0.01	−18.4	−0.21±0.01	−33.6

Note: The *t* values are all highly significant ($p < 0.0001$).

Statistical analysis

Overlapping temperature measurements from continuous recorders and the discrete measurements during 26 March – May 1998 were analyzed with Wilcoxon's matched-pairs test, to determine whether the two methods yielded different results. Mean water temperature for Yolo Bypass and the Sacramento River during the primary period of floodplain inundation (February–March) was analyzed with a generalized linear model with a variance function that increased with the mean squared, since variances were not homogeneous (Venables and Ripley 1997). Salmon FL measurements for Yolo Bypass and the Sacramento River during February–March of 1998 and 1999 were compared with a robust iteratively reweighted least squares regression procedure ("rlm"; Venables and Ripley 1997), because we detected substantial numbers of outliers in preliminary graphical evaluations of the data. Initial analyses revealed a substantial difference in the effects of location between years, so years were analyzed separately. Results from the CWT and bioenergetic studies were analyzed using a factorial-design analysis of variance, to evaluate the effects of location (Yolo Bypass, Sacramento River) and year (1998, 1999). Residuals from each model were examined graphically, to confirm that they met the assumption of normality and homogeneity of variance. Cochran and Levene's tests were also used, to test the assumption of homogeneity of variance. Logarithmic transformation was performed where necessary.

Results

Physical conditions

Yolo Bypass was inundated in 1998 and 1999 but the hydrology was substantially different in the two years (Fig. 2).

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Table 2. Results of salmon collections at Chipps Island for 1998 and 1999 coded-wire-tagged groups released concurrently in Yolo Bypass and the Sacramento River.

	1998		1999	
	Yolo Bypass	Sacramento River	Yolo Bypass	Sacramento River
Fork length (mm)	93.7±2.0	85.7±1.4	89.0±2.6	82.1±1.7
Migration time (days)	46.2±2.3	55.4±3.5	58.2±2.8	58.6±4.1
Apparent growth rate (mm·day^{-1})	0.80±0.06	0.52±0.02	0.55±0.06	0.43±0.03
Survival index	0.16	0.09	0.09	0.07
Sample size	9	10	9	8

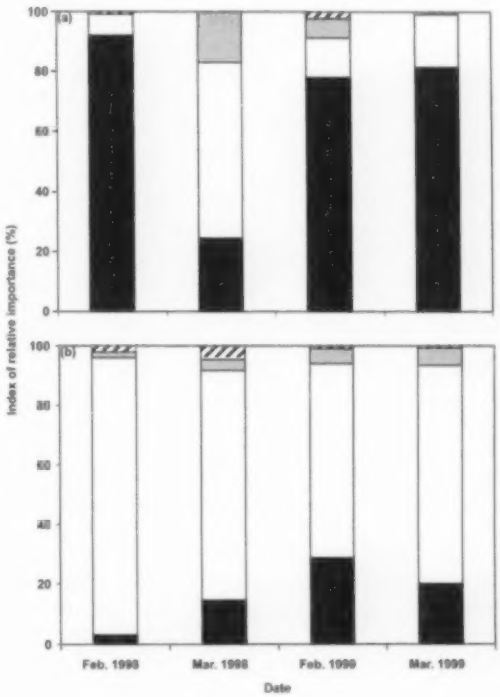
Note: Values for FL, migration time, and apparent growth rate are mean ± standard error (SEM).

The first year was extremely wet, with multiple flow pulses and a peak flow of 7200 $m^3 \cdot s^{-1}$. In 1999, floodplain hydrology was more moderate, with a peak of 1300 $m^3 \cdot s^{-1}$. Flows in the Sacramento River were much less variable than in the floodplain and generally remained at or below 2000 $m^3 \cdot s^{-1}$, a level within the design capacity (3100 $m^3 \cdot s^{-1}$) of the channel. Overlapping sampling between the continuous-temperature recorders and the discrete measurements during March–May 1998 showed a mean difference of 0.9°C between the two approaches, but this disparity was not statistically significant (Wilcoxon's matched-pairs test, $p > 0.25$). In 1998 and 1999, temperatures increased fairly steadily throughout the study period; however, in both years, temperature levels in Yolo Bypass were up to 5°C higher than those in the adjacent Sacramento River during the primary period of inundation, February–March. Temperature in the Yolo Bypass was described in 1998 by $T_y = -7.7 \pm 2.1 + (1.9 \pm 0.2)T_s$ and in 1999 by $T_y = -3.5 \pm 1.2 + (1.5 \pm 0.1)T_s$, where T_y is the temperature of the Yolo Bypass, T_s is the temperature of the Sacramento River, and the range for each value is the 95% confidence limit.

Fish growth, migration time, apparent growth rate, and survival

Salmon increased in size substantially faster in the Yolo Bypass than in the Sacramento River during each of the study years (Fig. 2). Robust regression results showed that the effect of location was highly significant ($p < 0.00001$) in each year (Table 1). This result is consistent with the CWT data (Table 2), which showed that the 1998 and 1999 Yolo Bypass CWT release groups had significantly larger mean length ($F = 14.34$, $p = 0.0006$) and higher apparent growth rates ($F = 20.67$, $p = 0.0007$) than the Sacramento River release groups. There was also a statistically significant effect of year: both release groups had larger mean sizes ($F = 4.42$, $p = 0.04$) and higher apparent growth rates ($F = 16.47$, $p = 0.0002$) in 1998 than in 1999. The 1998 Yolo Bypass CWT group showed the fastest migration time, arriving an average of at least 9 days ahead of any other release group. However, there was no statistically significant ($F = 2.22$, $p = 0.15$) effect of release location on migration time in the analysis of variance (ANOVA). As for fish size and apparent growth rate, mean migration time was slower in 1999 than in 1998 ($F = 5.60$, $p = 0.02$). There was no statistically significant interaction between location and year for salmon size ($F = 0.07$, $p = 0.78$), apparent growth rate ($F = 1.62$, $p = 0.21$), or migration time ($F = 1.8$, $p = 0.18$). The survival indices were somewhat higher for CWT groups released in the Yolo Bypass than for those released in the Sacramento River for both 1998 and 1999. However, the lowest coefficient of variation based on a Poisson distribution of the CWT recaptures is 32%, and the actual (unknown) distribution of counts is likely to have higher variance than a Poisson distribution. Clearly the confidence limits of the paired survival indices would overlap, so the differences are not statistically significant.

Fig. 3. Chinook salmon diet during February and March of 1998 and 1999 in Yolo Bypass (*a*) and the Sacramento River (*b*). The index of relative importance (*y*-axis) is defined in the text. Diptera (solid bars), zooplankton (open bars), other aquatic prey (shaded bars), and other terrestrial prey (striped bars) are shown for each month.



Diet

The diet of young salmon in the Yolo Bypass was dominated by dipterans, principally chironomid pupae and adults (Fig. 3). The second most common prey item was zooplank-

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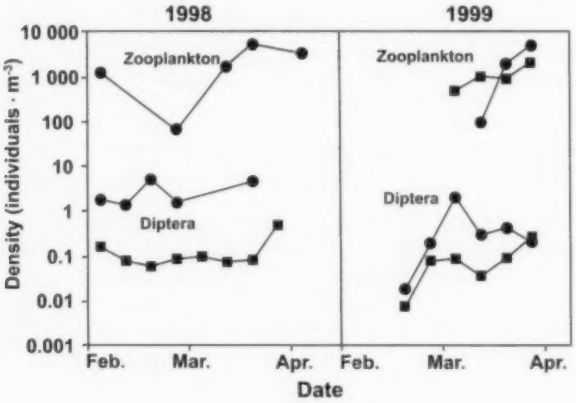
Fig. 4. Log_{10}-scaled weekly abundance (individuals·m^{-3}) of zooplankton and Diptera in Yolo Bypass (circles) and the Sacramento River (squares) during 1998 and 1999. Note that 1998 zooplankton data were not available for the Sacramento River.



ton, mostly cladocerans and copepods. Except for March 1998, zooplankton comprised less than 15% of the Yolo Bypass diets. Other aquatic (mainly amphipods and collembola) and terrestrial (mainly ants and arachnids) prey were relatively minor diet items. As for the floodplain samples, dipterans and zooplankton comprised over 90% of the diets of Sacramento River salmon; however, zooplankton were the dominant prey item in all months. Other aquatic (mostly amphipods, oligochaetes, and collembola) and terrestrial (mostly ants and other terrestrial insects) prey were consumed infrequently.

Prey availability

The drift samples contained many of the same taxa observed in the salmon diets, with Diptera (principally chironomids) as the major type at both sampling locations. However, the density of Diptera was much higher in the Yolo Bypass than in the Sacramento River (Fig. 4), particularly in 1998, when densities were consistently an order of magnitude higher. In general, dipteran drift densities were higher at each location in 1998 than in 1999. There was little difference in zooplankton density in the Yolo Bypass between 1998 and 1999 or between Yolo Bypass and the Sacramento River in 1999.

Bioenergetics

Young salmon from the Yolo Bypass had higher total-prey weights ($F = 39.2$, df = 1, $p < 0.0001$) than those from the Sacramento River (Fig. 5). The bioenergetic-modeling results showed that Yolo Bypass salmon also had higher wet weight ration indices than those from the Sacramento River ($F = 19.3$, df = 1, $p < 0.0001$). The interaction between location and year was significant for both the wet weight ration indices ($F = 10.0$, df = 1, $p = 0.02$) and the prey weights ($F = 4.7$, df = 1, $p = 0.03$).

Discussion

Chinook salmon that rear in the Yolo Bypass floodplain have higher apparent growth rates than those that remain in the adjacent Sacramento River channels. Mean length increased faster in the Yolo Bypass during each study year, and CWT fish released in the Yolo Bypass were larger and had higher apparent growth rates than those released in the Sacramento River. It is possible that these observations are due to higher mortality rates of smaller individuals in the Yolo Bypass or of larger individuals in the Sacramento River; however we have no data or reasonable mechanism to support this argument.

Apparent growth differences between the two areas are consistent with water temperature and stomach-content results. We found that the Yolo Bypass floodplain had significantly higher water temperatures and that young salmon from the floodplain ate significantly more prey than those from the Sacramento River. The wet weight ration indices calculated from bioenergetic modeling suggest that the increased prey availability in Yolo Bypass was sufficient to offset increased metabolic requirements from higher water temperatures. Higher water temperatures in the Yolo Bypass are expected as a result of the shallow depths on the broad floodplain. Increased feeding success in the Yolo Bypass is consistent with trends in prey availability. While Yolo Bypass and the Sacramento River had similar levels of zooplankton, Yolo Bypass had more dipteran prey in the drift, particularly in 1998. Studies of juvenile chinook salmon diets by Rondorf et al. (1990) showed that zooplankton were the least-favored prey items. Therefore, the dominance of zooplankton in the diets of Sacramento River salmon probably reflects a relatively low availability of other more energetically valuable prey items.

Recoveries of paired releases were too few to determine whether the higher survival indices for the Yolo Bypass release groups represent actual survival differences or random variation. Additional validation is needed from new release studies and from CWT recoveries in the adult ocean fishery and escapement. Nonetheless, the hypothesis that floodplain rearing could improve survival is substantiated by the growth data and bioenergetic modeling. Faster growth rates reflect improved habitat conditions, which would be expected to lead to improved survival, both during migration and later in the ocean. Elevated Yolo Bypass survival rates are also consistent with significantly faster migration rates in 1998, the likely result of which would be reduced exposure time to mortality risks in the delta, including predation and water diversions.

Improved survival is consistent with other habitat differences between the Yolo Bypass floodplain and the Sacramento River channel. We estimate that complete inundation of the Yolo Bypass creates a wetted area approximately 10 times larger than the reach of the Sacramento River we studied. This level of inundation is equivalent to a doubling of the wetted area of the entire delta portion of the San Francisco Estuary. Much of the floodplain habitat consists of broad shoals composed of soil and vegetation that are typical of the low-velocity conditions selected by young salmon (Everest and Chapman 1972). An increase in rearing area should reduce competition for food and space and perhaps reduce the probability of encountering a predator. In contrast, the Sacramento River channel is relatively narrow, with steep rock-reinforced banks and little shallow habitat. Migration through the Yolo Bypass corridor would also prevent

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fish from entering the channels of the central delta, in which there are various risks, including major water diversions (Brandes and McLain 2001). However, the Yolo Bypass is a less-stable environment, with stranding risks when flood waters recede. The relatively well-drained topography of the Yolo Bypass floodplain may help to reduce the magnitude of this problem. This is not to say, however, that access to floodplain rearing habitat represents the only mechanism to account for possible improvements in juvenile salmon survival in wetter years. Other covariates, such as reduced water temperature (Baker et al. 1995), reduced predation losses from higher turbidity (Gregory and Levings 1998), and reduced water diversion effects (Kjelson et al. 1982), also contribute to improved wet-year survival of salmon that migrate through the San Francisco Estuary.

The results from this study suggest that hydrology may affect salmon feeding success, migration, and survival in both floodplain and river habitat. The CWT results indicate that salmon grew faster, migrated faster, and may have had better survival rates in 1998 than in 1999. One clear difference between the years is that the flow pulses were higher and of longer duration in 1998 than in 1999. Higher flow could directly increase migration rates through higher water velocities and have multiple indirect effects on growth through factors such as food supply or water temperature. The abundance of Diptera in drift samples was substantially higher in 1998 than in 1999 in both locations. The significant interaction between location and year for both prey weights and the wet weight ration index indicates that the combined effects of diet and water temperature under 1998 hydrology should have resulted in higher growth rates. Higher growth rates and faster migration times in 1998 may, in turn, have improved survival by reducing predation risk. Higher-flow conditions in 1998 increased the quantity and duration of floodplain rearing area, perhaps reducing resource competition and predator encounter rates. Increased flow duration and magnitude in 1998 could also have improved survival on the floodplain by reducing stranding risks.

These results provide new insight into the significance of seasonal floodplain habitat for salmon rearing, which has been studied primarily in perennial waterways such as estuaries and rivers (Healey 1991; Kjelson et al. 1982). Indeed, this is the first study we are aware of demonstrating that off-channel floodplain provides major habitat for chinook salmon. We do not believe that the benefits of the floodplain to chinook salmon are unique to Yolo Bypass. Initial results from the Cosumnes River, an undammed watershed in the delta, show similar growth enhancements for juvenile chinook salmon that rear on the floodplain rather than in adjacent river channels (Peter Moyle, University of California, Davis, CA 95616, personal communication). Moreover, the benefits of the floodplain to salmon are consistent with findings for other fish species. Sommer et al. (1997) found that the Yolo Bypass provides major spawning, rearing, and foraging habitat for the native cyprinid Sacramento splittail (*Pogonichthys macrolepidotus*). The spawning and rearing of fish on floodplains has been reported in diverse locations that range from small streams (Halyk and Balon 1983; Ross and Baker 1983) to large rivers (Copp and Penaz 1988) in both temperate (Gehrke 1992; Turner et al. 1994) and tropical (Winemiller and Jepsen 1998) locations. The growth effects of floodplain habitat have been described for several tropical locations (Welcomme 1979); however, the present study and the results of Gutreuter et al. (2000) represent the only examples from temperate rivers of which we are aware.

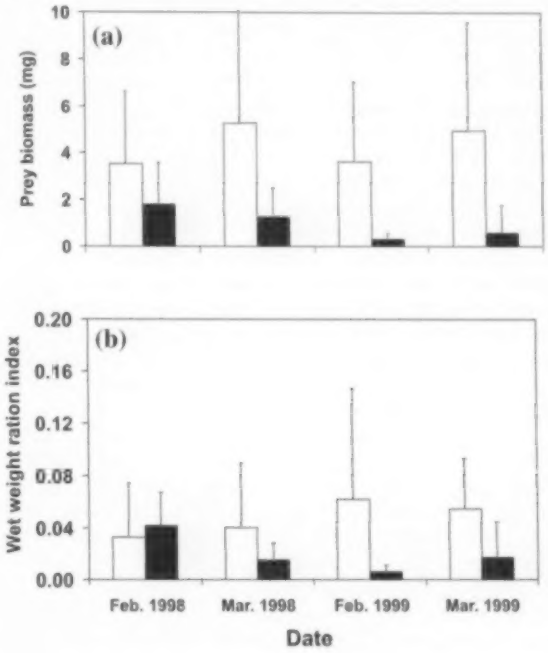

Fig. 5. Feeding success results for Yolo Bypass (open bars) and Sacramento River (solid bars) juvenile salmon during 1998 and 1999. (*a*) Estimated prey weights in stomach contents. (*b*) Wet weight ration indices. Means and standard errors are shown.

Differences between the invertebrate communities in floodplains versus river channels have been reported by Castella et al. (1991). The exceptional production of drift invertebrates on the Yolo Bypass floodplain is consistent with the results of Gladden and Smock (1990), who found that invertebrate production was one to two orders of magnitude greater on the floodplain than in adjacent streams. Although we did not monitor benthic invertebrates, results from other studies of large rivers indicate that benthic biomass may be up to an order of magnitude higher in the floodplain (Junk et al. 1989). The Yolo Bypass drift invertebrate results contrast with the results for zooplankton, which were not particularly abundant on the floodplain. This finding is comparable with that of Welcomme (1979), who reported that densities of zooplankton in natural floodplains are frequently low, except for low-water periods and localized concentrations near habitat interfaces such as shorelines.

The mechanism for greater abundance of drift invertebrates in the Yolo Bypass remains unclear, but is unlikely to be an artifact of land use on the floodplain. Possible explanations for increased drift abundance include increased food supply (e.g., primary production or detritus), more habitat, and longer hydraulic residence times. For each of these mechanisms, Yolo Bypass probably provides functions similar to more "natural" floodplains. Improved food supply is supported by the work of Jassby and Cloern (2000), whose

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modeling studies suggest that the Yolo Bypass should have enhanced phytoplankton production as a result of its large surface area and shallow depth. Inputs of fertilizers from agriculture in the Yolo Bypass would not be important contributing factors, as nitrogen and phosphorous are rarely limiting to phytoplankton production in the delta (Ball and Arthur 1979). Like less-disturbed floodplains in other regions (Junk et al. 1989), invertebrate production in the Yolo Bypass may be stimulated by an increased availability of detritus in the food web. Alternatively, the trends in invertebrate abundance we observed may be a consequence of physical differences between floodplain and channel habitat. Inundation of the floodplain may increase the amount of habitat for benthic invertebrates, a major source of drift biomass. Given the larger surface area and lower velocities in Yolo Bypass, the floodplain probably has a much longer hydraulic residence time than the Sacramento River, reducing the rate at which drift invertebrates would be flushed out of the system. Increased habitat area and hydraulic residence time would also have been functional characteristics of the historical floodplain.

In the broader context, the results for salmon and drift invertebrates are consistent with the flood pulse concept, which predicts that floodplains should yield greater fish and invertebrate production than channel habitat (Junk et al. 1989). This finding is significant in that the flood pulse concept was developed primarily on the basis of relatively undisturbed rivers, whereas our study was conducted in a regulated river with a floodplain dominated by agricultural uses. Gutreuter et al. (2000) showed similar enhancements in fish growth from floodplain inundation in the Upper Mississippi River, another large regulated river. These studies suggest that floodplains can maintain important functional characteristics even in heavily modified rivers. In the case of the San Francisco Estuary and its tributaries, we do not claim that floodplain inundation is the primary factor regulating the productivity of the system. The Yolo Bypass floodplain may be seasonally more productive than the Sacramento River for some fish and invertebrates, but we have no data regarding its contribution during dry months or years. Nonetheless, the results of the present study and of Sommer et al. (1997) are sufficient to demonstrate that the floodplain represents one of the most biologically important habitat types in the region. We believe that proposed large-scale restoration activities in the San Francisco Estuary and its tributaries (Yoshiyama et al. 2000) that would increase the area and connectivity of the floodplain offer particular promise for native fish populations such as chinook salmon and Sacramento splittail.

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A catch per unit effort – soak time model for the Bristol Bay red king crab fishery, 1991–1997

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Abstract: Postseason commercial fisheries data are used to estimate a catch per unit effort (CPUE) – soak time relationship for the 1991–1993 and 1996–1997 Bristol Bay red king crab (*Paralithodes camtschaticus*) fishery in order to gain regulatory and in-season management insight. Use of commercial fishery data allows our model to capture the influence of biological and environmental effects as well as behavioral responses of crabbers to changing natural and regulatory conditions on CPUE. However, data deficiencies present a variety of estimation challenges, especially when the data are derived from neither a contemporaneous nor a scientifically designed sample of the fleet. A statistical framework for dealing with such challenges is illustrated in this paper. This research uncovered three major results. First, data pooling guided by recursive estimation/hypothesis testing is shown to be essential. Second, the analysis provides insight into CPUE response to changing conditions, whether biological, ecological, or policy induced. Third, it is apparent that more complete and contemporaneous collection of commercial fisheries data is critical to refine the estimation of CPUE – soak time relationships. Then, it may be possible to isolate the inter- and intra-seasonal influence of biological and environmental effects as well as behavioral responses of crabbers to changing natural and regulatory conditions.

Résumé : Des statistiques de la pêche commerciale au Crabe royal (*Paralithodes camtschaticus*) compilées après la saison de pêche à Bristol Bay en 1991–1993 et 1996–1997 ont servi à estimer la relation entre la capture par unité d'effort de pêche (CPUE) et la durée de mouillage de l'équipement, afin d'obtenir une meilleure évaluation de la réglementation et de la gestion pendant la saison. Les données de pêche commerciale permettent au modèle de capter l'influence sur la CPUE des facteurs biologiques et environnementaux, ainsi que des réactions comportementales des pêcheurs aux conditions changeantes du milieu naturel et de la réglementation. Cependant, des carences dans les données causent plusieurs problèmes d'estimation, particulièrement lorsque les données proviennent d'un échantillonnage de la flotte qui n'est ni concomitant, ni scientifique; nous faisons la démonstration d'un cadre statistique qui permet de résoudre de tels problèmes. Trois conclusions principales s'imposent. D'abord, il est essentiel que le regroupement des données soit fait à la suite d'estimations récursives et d'épreuves d'hypothèses. Ensuite, l'analyse permet de percevoir les effets sur la CPUE de changements de conditions, qu'ils soient de nature biologique ou écologique ou qu'ils soient reliés à la politique de réglementation de la pêche. Finalement, il ressort que la récolte de données plus complètes et plus concomitantes sur la pêche commerciale est essentielle pour améliorer l'estimation de la relation entre la CPUE et la durée de mouillage. Il sera alors peut-être possible de séparer les composantes intrasaisonnières et intersaisonnières des effets des facteurs biologiques et environnementaux et des réactions comportementales des pêcheurs aux changements du milieu naturel et de la réglementation de la pêche.

[Traduit par la Rédaction]

Introduction

The U.S. Bering Sea crab fisheries developed rapidly throughout the 1970s following the decline of stocks out of Kodiak Island. The Bristol Bay red king crab (*Paralithodes camtschaticus*) fishery collapsed after peaking at a harvest of 58 968 tons in 1980. The fishery was closed for 1 year in 1983. Since then, Bristol Bay harvests have remained relatively low and two additional closures occurred in 1994 and 1995.

The Bristol Bay red king crab fishery is a license-limited open-access fishery managed by the Alaska Department of Fish and Game. Management authority is delegated to the Alaska Department of Fish and Game by a federal fishery management plan, the goal of which is to maximize the overall long-term benefit to the nation of the Bering Sea king crab stocks. Annual trawl surveys conducted by the National Marine Fisheries Service provide stock abundance data. The Alaska Department of Fish and Game analyzes the data and, in consultation with the National Marine Fisheries Service, recommends to the Alaska Board of Fisheries a preseason quota or guideline harvest level (GHL), measured in pounds of crab, that the estimated biomass can support. The Alaska Department of Fish and Game then uses in-season fishery performance, including legal crabs caught per pot lift or catch per unit effort (CPUE) and an estimate of cumula-

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tive catch to close the fishery at the GHL (Herrmann and Greenberg 1997).

Fishery management has become increasingly difficult as depressed stocks and lower exploitation/utilization rates shortened seasons. Managers implemented a uniform pot limit of 250 pots per vessel in 1992 in an attempt to elongate the season by mitigating fleet fishing power. This policy was challenged by industry as discriminatory toward large vessels. A two-tiered pot limit was implemented in 1993, where vessels less than or equal to 38.10 m in length were allowed to fish 200 pots, while vessels over 38.10 m were limited to 250 pots. Nevertheless, season length compressed from 9 days in 1991 to 4 days by 1996, when harvest exceeded the preseason GHL by 68% and the fishery experienced its largest CPUE (16 crabs per pot lift) since the fishery began to collapse in 1981. Managers reacted by halving the pot limit in 1997. Harvest once again exceeded the GHL, this time by 25%. Herrmann and Greenberg cited underestimation of CPUE as a principal cause of the excessive harvest.

Understanding the CPUE – soak time relationship is important to managing this vulnerable but lucrative crab fishery (Pengilly and Tracy 1998). The purpose of this paper is to use commercial fisheries data to estimate a CPUE – soak time relationship for the Bristol Bay red king crab fishery to gain regulatory and in-season management insight. The analysis is conducted using postseason commercial fisheries data for the 5 years the fishery was open, 1991–1993 and 1996–1997. The use of commercial fisheries data distinguishes our paper from the experiment-based literature that focuses on stock assessment. It allows our model to capture the aggregate influence of biological and environmental effects as well as behavioral responses of crabbers to changing natural and regulatory conditions on CPUE. Use of commercial fisheries data, however, presents a variety of estimation challenges, especially when the data are derived from neither a contemporaneous nor a scientifically designed sample of the fleet. A statistical framework for dealing with such challenges is illustrated in this paper.

Methods

Model specification

The relationship between CPUE and soak time is extensively reviewed in Miller (1990), primarily in the context of using CPUE as a method of stock assessment. Collectively, the literature provides evidence that CPUE (generally measured as total catch of crabs or fish per trap) increases asymptotically with soak time due to a progressive reduction in both bait effectiveness and the density of the species being fished. Additional factors found in the experiment-based literature to affect the shape of the asymptotic CPUE – soak time relationship include size and effectiveness of the gear (e.g., Munro 1974), inter- and intraspecies behavioral interactions (e.g., Miller 1978), local stock density (e.g., Sinoda and Kobayasi 1969), and general environmental conditions such as temperature, time of day, and current (e.g., Bennett and Brown 1979).

An asymptotic catch model is particularly appropriate to describe the CPUE – soak time relationship for short fishing seasons where catches are not observed to decline much with soak time (Zhou and Shirley 1997). Such is the case with the Bristol Bay red king crab fishery. The study by Pengilly and Tracy (1998) specifically supports the use of an asymptotic catch model for Bristol Bay red king crab. They conducted a controlled experiment to assess the effects of soak time on catch of legal-sized red king crabs and by-catch of nonlegal crabs in Bristol Bay. While their study was descriptive and made no attempt to estimate an asymptotic catch model, their analysis provided evidence of an asymptotic CPUE – soak time relationship for Bristol Bay red king crabs.

Specification of an asymptotic catch model for the Bristol Bay red king crab fishery theoretically should incorporate many of the factors found in the experiment-based literature that capture both intra- and inter-seasonal variations. Unfortunately, data limitations prevent isolating likely factors that define CPUE – soak time relationships within and across seasons. Accordingly, the approach taken in this study is to model CPUE – soak time in a way that encompasses the basic asymptotic relationship within a more general specification that captures aggregate or composite intra- and interseasonal variations, whatever the cause: biological, environmental, or behavioral. Three models, listed in order of hierarchical nesting, are specified in eqs. 1, 2, and 3.

Equation 1, the most common asymptotic catch model, is a von Bertalanffy type equation:

$$(1) \qquad C_i = \beta_0(1 - e^{(-\beta_2 ST_i)}) + \varepsilon_i$$

where C_i denotes CPUE observations, $i = 1,..., n$; ST_i denotes observations on soak time, measured in days; β_0 and β_2 are unknown parameters (scalars) representing, respectively, the asymptotic catch and the rate at which it is reached; and ε_i denotes an error term.

Theoretical considerations suggest that eq. 1 should be generalized so that asymptotic catch depends on the initial stock. It should also reflect the possibility that the rate at which a pot fishes toward its maximum may depend on localized stock depletion throughout the fishing season. That is, a particular CPUE – soak time relationship, on a given day of the season, is a function of residual biomass and thus past fishing behavior. Data limitations prevent estimating such a specification. Instead, eq. 2 incorporates an aggregate intraseasonal variable, drop time (DT), which is defined as the day within the season when a pot is set into the water. This variable proxies for all factors contributing to changing catch as the season progresses. It accounts for the fact that pots dropped at the beginning of the fishing season are exposed to a more abundant, denser stock of crabs than pots dropped at the end of the fishing season. We have

$$(2) \qquad C_i = \beta_0(1 + \beta_1 DT_i)(1 - e^{(-\beta_2(1+\beta_3 DT_i)ST_i)}) + \varepsilon_i.$$

The new parameters represent the effect of drop time on the asymptotic catch level, β_1, and the effect of drop time on the rate at which a pot fishes toward this asymptote, β_3. Interpretation of β_0 and β_2 is unchanged. Note that the elementary asymptotic catch model given by eq. 1 is embedded in the asymptotic catch model expressed by eq. 2, i.e., eq. 1 is a special case of eq. 2, where $\beta_1 = 0$ and $\beta_3 = 0$.

A principal cause of interseasonal variation in the asymptotic catch model is due to different initial biomasses across years. Numerous other interseasonal factors conceivably impact both the catch rate and the asymptote. Spatial distribution of crab varies across years, as do tidal conditions or other environmental factors that affect recruitment into the gear. Regulatory conditions under which the Bristol Bay red king crab fishery operated also changed throughout 1991–1997. Fishery managers imposed pot limits in 1992 and reduced the exploitation rate from 20 to 10% in 1996 and 1997. Fishing strategies adjust to all of these factors.

Equation 3 introduces a fishing season indicator variable (FS) into eq. 2 in order to capture generic, aggregate seasonal effects:

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$$(3)\quad C_i = \sum_{j=91}^{97} \beta_{0,j} FS_{i,j} \left(1 + \left(\sum_{j=91}^{97} \beta_{1,j} FS_{i,j}\right) DT_i\right) \times \left(1 - e^{-\left(\sum_{j=91}^{97} \beta_{2,j} FS_{i,j}\right)\left(1 + \left(\sum_{j=91}^{97} \beta_{3,j} FS_{i,j}\right) DT_i\right) ST_i}\right) + \varepsilon_i .$$

The indicator variable $FS_{i,j}$ attributes pot lifts to the fishing season in which they occurred, j = 91, 92, 93, 96, and 97, i.e., $FS_{i,j}$ = 1 if pot lifts i occurred in season j and 0 otherwise. The principal attribute of model 3 is that it introduces flexibility by allowing the coefficients β_0, β_1, β_2, and β_3 to take on year-specific values. For example, β_2 could take the value $\hat{\beta}_{2,91}$ in 1991, $\hat{\beta}_{2,92}$ in 1992, $\hat{\beta}_{2,93}$ in 1993, and so on. Equation 3 specifies year-specific asymptotic CPUE – soak time models that account for intraseasonal variations similar to eq. 2. In the absence of any parameter restrictions, estimating eq. 2 separately for each year is equivalent to estimating eq. 3. Estimation of both models 2 and 3, together with an examination of statistically admissible parameter restrictions, was pursued in order to determine the type of asymptotic catch model applied to all five fishing seasons.

Data considerations

The commercial fishery data used in this study are drawn from Alaska Department of Fish and Game after-season dockside interviews with available catcher vessels and from contemporaneous weekly trip summaries completed by observers on catcher–processors (CPs). These surveys provide information on vessels' daily performances. Variables of interest include day fished, the number of pots lifted on that day, the total number of crabs caught, and the average soak time of the pots lifted. Twenty-two to 48% of the catcher vessel fleet was surveyed during the five seasons between 1991 and 1997. The number of CP vessels — all surveyed — ranged from 0 to 24. Missing data on the variables of interest rendered 7–57% of all surveys unusable, depending on the year. The sampled data are not the result of a statistical design that was intended to represent a randomized cross section of a heterogeneous fleet. Nor do most of the usable surveys provide information on vessels' actual daily performances; 47–86% of the observations across the seasons are not contemporaneous records of daily fishing activities but appear to be seasonal averages. Depending on year, 20–31% of the usable catcher vessel surveys recorded data for only a single day or recorded the same catch every day. An additional 27–59% of the usable surveys do not reflect different levels of catch every day, as would be expected of contemporaneous catch records. Surprisingly, 37–88% of the usable CP surveys also report identical catch records on adjacent days, despite the fact that CP data are collected by onboard observers.

These data deficiencies pose problems for model estimation. Rather than eliminating noncontemporaneous data, data from all complete surveys were utilized after they were first aggregated. The aggregation was done on a year-by-year basis such that observations with identical soak times on the same day were aggregated together to form a single, final observation. Thus, year-specific CPUE performances were averaged out over observations of pots pulled on a given day that soaked the same length of time. This data aggregation scheme isolated all reported day – soak time combinations, thereby retaining all intraseasonal CPUE – soak time variation across the surveyed portion of the fleet.

The aggregation process is illustrated in Tables 1 and 2. The aggregated data corresponding to Table 1 are shown in Table 2. In Table 1, vessels X and Y both recorded pulling pots on day 2, and both utilized a 2-day soak. These two observations are collapsed into one, indicated as observation No. 1 in Table 2, which reflects the total number of pots lifted and crabs harvested on day 2, given a 2-day soak. In this illustration, 2400 crabs were caught by 200 pots. The drop time variable is derived by subtracting soak time from the day fished; CPUE is calculated as the aggregate number of pots lifted divided by the aggregate number of crabs caught. For example, the first observation in Table 2 is based on pots dropped at the start of the fishing season (drop time 0) and is calculated to have an average CPUE of 12 crabs.

Table 1. Initial daily observations (illustrative sample).

Vessel identification letter	Day fished	Number of crabs caught	Number of pots lifted	Pot soak time (days)
X	2nd	1600	120	2
X	3rd	1500	120	3
X	4th	900	80	2
Y	2nd	800	80	2
Y	3rd	900	80	3
Z	2nd	1100	100	1

The final aggregated data set totaled 235 observations over the five open seasons, 1991–1993 and 1996–1997. Aggregated CPUE observations are based on daily catch records of 1–69 vessels. An observation represents the average CPUE from as few as 12 and as many as 11 816 pots that were dropped on a particular day and that soaked an identical amount of time.

The range and variation of soak times over which catches are observed in commercial fishery data are dictated by fishing conditions and strategies. This potentially adds further difficulty to the estimation process because neither the data range nor the variation may be large enough to uncover an asymptotic catch model, even if true.

The soak time frequency distribution reported in the available data is presented in Table 3. The range of soak times covered by the data varies across years but is especially short in 1997, when 81.7% of the pots soaked at most 1 day and no pot soaked longer than 2 days. Variation in soak times was the smallest in 1997, with a mean soak time of 0.96 day and a standard deviation of 0.29. It follows that the estimation of a CPUE – soak time model based on individual year data such as eq. 2 is likely to fail for 1997. An alternative estimation approach capable of coping with this common characteristic of commercial fisheries data is to pool data across years.

Data pooling shares information between 1997 and other seasons, increasing the range and variation in soak times observed in the expanded data set, thereby enhancing the likelihood of identifying an asymptotic catch model for that year. Data pooling is accomplished by imposing one or more cross-year parameter restrictions on model 3. For example, the rate at which a pot filled in 1997 might be statistically identical to that of 1996, allowing the coefficient β_2 to share a common value in 1996 and 1997. In such a situation, the following restriction might be imposed on model 3: $\beta_{2,96} = \beta_{2,97}$. Of course, any such restrictions would need to be tested for statistical validity.

Estimation

Data pooling in the context of model 3 can have negative consequences. There is always the risk that restrictions imposed by data pooling might be incorrect (Type II error). Moreover, the more restrictions tested based on the information in one data set, the greater the potential for compounding Type II errors. Sequential statistical tests of the restrictions are thus not sufficient; a benchmark is also needed to evaluate the validity of the final restrictions imposed on model 3. Model 2 provides such a reference point.

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Table 2. Final aggregated observations (illustrative sample based on Table 1).

Observation	Day fished	Number of crabs caught	Number of pots lifted	Pot soak time (days)	Pot drop time (days)	Catch per pot
1st	2nd	2400	200	2	0	12
2nd	3rd	2400	200	3	0	12
3rd	4th	800	80	2	2	10
4th	2nd	1100	100	1	1	11

Table 3. Soak time frequency distribution across fishing seasons.

Year	Number of final aggregated observations	Final sampled vessels[a]	Mean CPUE	Mean soak time in days[b]	Number of pots lifted with reported soak time of:[c] ≤1 day	1–2 days	2–3 days	>3 days
1991	55	104 (34%)	12	1.98 (0.54)	10 053 (13.0%)	58 297 (75.2%)	7969 (10.3%)	1158 (1.5%)
1992	26	36 (13%)	5	1.28 (0.48)	20 195 (70.7%)	8 241 (28.8%)	74 (0.3%)	60 (0.2%)
1993	41	122 (42%)	8	1.63 (0.58)	37 664 (41.1%)	50 423 (55.1%)	2920 (3.2%)	536 (0.6%)
1996	52	70 (36%)	18	1.13 (0.30)	17 639 (64.8%)	9 506 (34.9%)	90 (0.3%)	0 (0%)
1997	61	50 (19%)	17	0.96 (0.29)	13 633 (81.7%)	3 054 (18.3%)	0 (0%)	0 (0%)

[a]As percentage of fleet in parentheses.

[b]SD in parentheses. Mean soak times are weighted by the number of pot lifts per observation. Mean soak time in year j is $\overline{ST}_j = \frac{\sum_{i=1}^{n_j} m_i ST_i}{\sum_{i=1}^{n_j} m_i}$ and SD is $s_j = \sqrt{\frac{\sum_{i=1}^{n_j} m_i (ST_i - \overline{ST}_j)^2}{\sum_{i=1}^{n_j} m_i - 1}}$, where m_i denotes the number of pots on which observation i is based and n_j denotes the number of observations in year j.

[c]Number of pots lifted as percentage of total pots lifted from usable data of that year in parentheses.

Theoretically, year-specific parameter values estimated in model 2 should be identical to those of model 3, in the absence of data pooling. Accordingly, model 2 parameter estimates, for years in which asymptotic catch models are identified, are compared with corresponding model 3 parameter estimates. The restrictions imposed on model 3 are pertinent only if they are not statistically rejected. Moreover, they must result in parameter estimates that make logical sense compared with what is already known a priori about the CPUE – soak time relationships as well as what is learned from unconstrained and significant parameter estimates generated from model 2. That is, estimates of both models 2 and 3 should be close and consistent with one another for those parameters that are statistically significant and precisely estimated. In the final analysis, which of the models provides the better empirical representation of the CPUE – soak time relationship involves considerations of mean squared error, bias–variance trade-offs, and interpretability of the estimated relationship.

Two sources of prima facie heteroskedasticity were corrected prior to estimation: one source relates to unequal intraseasonal variances and the other relates to unequal interseasonal variances. Average CPUE observations are based on different numbers of pots pulled, which generally introduces prima facie intraseasonal heteroskedasticity across observations. In particular, CPUE observations based on averages of a large number of pots pulled generally have smaller variances than CPUE observations based on a small number of pots pulled, all other things being equal. This group-induced heteroskedasticity problem is a common artifact of combining individual data into aggregated averages. Heteroskedasticity arises when the data set contains an unequal number of observations across groups (Johnston 1984). For purposes of parameter estimation, the standard group-induced heteroskedasticity correction is introduced; data observations in eqs. 2 and 3 are weighted by the square root of the number of pot lifts in each observation. Specifically, both sides of the equations are multiplied by $\sqrt{m_i}$, where m_i is the number of pots lifted for observation i:

$$(2.1)\quad \sqrt{m_i}C_i = \sqrt{m_i}\left(\beta_0(1+\beta_1 DT_i)\left(1-e^{\left(-\beta_2(1+\beta_3 DT_i)ST_i\right)}\right)\right)+\varepsilon_i$$

$$(3.1)\quad \sqrt{m_i}C_i = \sqrt{m_i}\left(\sum_{j=91}^{97}\beta_{0,j}FS_{i,j}\left(1+\left(\sum_{j=91}^{97}\beta_{1,j}FS_{i,j}\right)DT_i\right)\times\left(1-e^{\left(-\left(\sum_{j=91}^{97}\beta_{2,j}FS_{i,j}\right)\left(1+\left(\sum_{j=91}^{97}\beta_{3,j}FS_{i,j}\right)DT_i\right)ST_i\right)}\right)\right)+\varepsilon_i.$$

Non prima facie sources of intraseasonal heteroskedasticity also may exist. For example, CPUE may exhibit smaller variance as it becomes less dependent on local crab density at increasing soak times and drop times. A Breush–Pagan–Godfrey heteroskedasticity test (Mittelhammer et al. 2000) was applied to eq. 3.1 and thus implicitly on the nested eq. 2.1. In particular, an auxiliary regression was specified expressing estimated squared residuals as a linear function of soak time and drop time. The hypotheses of homoskedasticity relative to variations in soak time and drop time could not be rejected at typical levels of Type I error probabilities (two-sided t distribution probability values were 0.21922 and 0.2009, respectively, for soak time and drop time).

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Table 4. Final model 2.1 specification and estimates.

Parameter	Definition		Year 1991	1992	1993	1996	1997
$\hat{\beta}_0$	Asymptotic catch	Estimated coefficient	23.4583	7.6122	12.4758	34.5322	5.6497
		SE	3.4579	0.8040	0.4654	3.1375	3.7145
		Two-sided t probability value	0.000	0.000	0.000	0.000	0.000
$\hat{\beta}_1$	Effect of drop time on the asymptotic catch	Estimated coefficient	–0.0761	–0.0425	–0.0770	–0.2023	0.0504
		SE	0.0095	0.0157	0.0059	0.0112	0.0844
		Two-sided t probability value	0.000	0.013	0.000	0.000	0.552
$\hat{\beta}_2$	Rate at which the asymptotic catch is reached	Estimated coefficient	0.6293	1.6134	2.7559	2.9879	0.7702
		SE	0.1929	0.4993	0.7030	1.7600	2.0921
		Two-sided t probability value	0.002	0.004	0.000	0.096	0.714
$\hat{\beta}_3$	Effect of drop time on the rate at which the asymptotic catch is reached	Estimated coefficient			–0.0473		2.8243
		SE			0.0329		8.4024
		Two-sided t probability value			0.159		0.738
		R^2	0.9281	0.8787	0.9579	0.8953	0.3292

CPUE observations are also subject to unequal interseasonal variances due to the different levels of biomass to which pots were exposed across years. Other potential seasonal effects (e.g., tides and weather) also may contribute to year-specific CPUE variances. Equation 3.1 is further transformed to correct for this prima facie interseasonal heteroskedasticity. The correction was implemented by multiplying both sides of the equation by $\frac{1}{\hat{\sigma}_{i,j}}$, where $\hat{\sigma}^2_{i,j}$ is the estimated variance of the residual terms for fishing season j; $\hat{\sigma}^2_{i,j} = \frac{\sum_{i \in \Phi_j} \hat{e}_i^2}{n_j - k}$ and $\hat{e}_i = \hat{C}_i - C_i$, where $\hat{C}_i$ is the CPUE value calculated from model 3.1, Φ_j denotes the set of observations corresponding to season j, n_j is the number of observations for fishing season j, and k is the number of parameters (Johnston 1984). The weighted model form is given in eq. 3.2:

$$(3.2)\quad \frac{1}{\hat{\sigma}_{i,j}}\sqrt{m_i}C_i = \frac{1}{\hat{\sigma}_{i,j}}\left(\sqrt{m_i}\left(\sum_{j=91}^{97}\beta_{0,j}FS_{i,j} \times \left(1+\left(\sum_{j=91}^{97}\beta_{1,j}FS_{i,j}\right)DT_i\right) \times \left(1-e^{-\left(\sum_{j=91}^{97}\beta_{2,j}FS_{i,j}\right)\left(1+\left(\sum_{j=91}^{97}\beta_{3,j}FS_{i,j}\right)DT_i\right)ST_i}\right)\right)\right) + \varepsilon_i.$$

Model 2.1, applied individually to each year, and model 3.2, applied to all years simultaneously, are estimated by nonlinear least squares using the GAUSS and Newton–Raphson algorithms contained in the GAUSSX statistics program (GAUSSX Econometric Software, 1996). For details regarding the GAUSS and Newton–Raphson algorithms, see Mittelhammer et al. (2000).

Results

Initial estimation of model 2.1 revealed evidence of an asymptotic catch model for all years but 1997. However, the results also indicated evidence of specification error in the sense of insignificant β_3 parameter estimates for three years. Equation 2.1 was respecified without the β_3 parameter in these cases and reestimated for the affected seasons, 1991–1992 and 1996. Final estimated models 2.1 are presented in Table 4. Table 4 shows well-defined asymptotic CPUE – soak time relationships for the years 1991–1993 and 1996. Not surprisingly, estimates for 1997, the year of little soak time variation, reveal no significant statistical evidence of an asymptotic catch model for that year.

Equation 3.2 was estimated in a three-step process with the goal of identifying an asymptotic CPUE – soak time relationship for 1997 while also maintaining a model structure for 1991–1993 and 1996 comparable with that presented in Table 4. The first step involved estimating model 3.2 with no data pooling, i.e., without shared-parameter restrictions. The second step involved testing for data pooling restrictions. The third step involved reestimating model 3.2, specified according to the nonrejected restrictions. Then, the estimated coefficients of model 3.2 for the years 1991–1993 and 1996 were compared with the corresponding estimates obtained from model 2.1 (Table 4).

Initial unrestricted estimates of model 3.2 revealed evidence of model misspecification for the years 1991–1992 and 1996, similar to the initial estimation of model 2.1. This finding suggested that any respecification of model 3.2 required, at the least, a restriction on the β_3 parameters such that $\beta_{3,91} = \beta_{3,92} = \beta_{3,96} = 0$. This restriction is used to delimit potential specifications of model 3.2 that might reveal an asymptotic catch model for 1997. All of the potential restrictions that were tested on model 3.2 are presented in Table 5, stated from the least to the most restrictive.

The null hypotheses were tested to determine which of the possible restrictions on the parameters, if any, should be imposed on the final model. The final pooled model 3.2 specification was chosen to be the least restrictive, statistically significant specification, in an attempt to retain as much of the year specificity outlined by model 2 estimates as possible. Final estimates are presented in Table 6, which indicate year-specific asymptotic catch relationships for all five fishing seasons.

Overall model fit is good. All the explanatory variable effects are statistically significant at standard levels of Type I error probabilities except one (two-sided t probabilities of 0.000 for all but two variables that have t probabilities of

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Table 5. Model 3.2 potential restrictions.

β_0, β_1, and β_2	β_3
$H_0 : \beta_{q,j} = \beta_{q,97}$	$H_0 : \beta_{3,91} = \beta_{3,92} = \beta_{3,96} = 0$
$H_0 : \beta_{q,j} = \beta_{q,l} = \beta_{q,97}$; $j \neq l$	$H_0 : \beta_{3,91} = \beta_{3,92} = \beta_{3,96} = 0$, $\beta_{3,93} = \beta_{3,97}$
$H_0 : \beta_{q,j} = \beta_{q,l} = \beta_{q,p} = \beta_{q,97}$; $j \neq l \neq p$	$H_0 : \beta_{3,91} = \beta_{3,92} = \beta_{3,96} = \beta_{3,97} = 0$
$H_0 : \beta_{q,91} = \beta_{q,92} = \beta_{q,93} = \beta_{q,96} = \beta_{q,97}$	

Note: j, l, p = 91, 92, 93, 96 and q = 0, 1, 2.

Table 6. Final model 3.2 specification and estimates.

Model specified by following restriction (F probability (df: 10, 219) = 0.3028):

H_0: $\beta_{0,91} = \beta_{0,97}$
H_0: $\beta_{1,91} = \beta_{1,92} = \beta_{1,93} = \beta_{1,97}$
H_0: $\beta_{2,93} = \beta_{2,96} = \beta_{2,97}$
H_0: $\beta_{3,91} = \beta_{3,92} = \beta_{3,96} = \beta_{3,97} = 0$

Parameter			Year 1991	1992	1993	1996	1997
$\hat{\beta}_0$	Asymptotic catch	Estimated coefficient	22.8094	8.2095	12.2619	34.3919	22.8094
		SE	1.3955	1.4423	0.3401	1.9936	1.3955
		Two-sided t probability value	0.000	0.000	0.000	0.000	0.000
$\hat{\beta}_1$	Effect of drop time on the asymptotic catch	Estimated coefficient	–0.0747	–0.0747	–0.0747	–0.2029	–0.0747
		SE	0.004	0.0044	0.0044	0.0125	0.0044
		Two-sided t probability value	0.000	0.000	0.000	0.000	0.000
$\hat{\beta}_2$	Rate at which the asymptotic catch is reached	Estimated coefficient	0.6586	1.8575	3.1289	3.1289	3.1289
		SE	0.0920	1.1761	0.6312	0.6312	0.6312
		Two-sided t probability value	0.000	0.116	0.000	0.000	0.000
$\hat{\beta}_3$	Effect of drop time on the rate at which the asymptotic catch is reached	Estimated coefficient			–0.0604		
		SE			0.0200		
		Two-sided t probability value			0.003		
		R^2					0.9306
		Mean absolute percent error (MAPE)					19.74
		Mean percent error (MPE)					–8.25
		Mean absolute deviation (MAD)					1.59

Note: MAPE, MPE, and MAD are weighted by the number of pots that each observation is based on MAPE $= \frac{100}{\sum_{i=1}^{n} m_i} \sum_{i=1}^{n} m_i \left| \frac{C_i - \hat{C}_i}{C_i} \right|$,

MPE $= \frac{100}{\sum_{i=1}^{n} m_i} \sum_{i=1}^{n} m_i \frac{C_i - \hat{C}_i}{C_i}$, and MAD $= \frac{100}{\sum_{i=1}^{n} m_i} \sum_{i=1}^{n} m_i \left| C_i - \hat{C}_i \right|$, where $\hat{C}_i$ is the CPUE value calculated from final model 3.2 estimates.

0.003 and 0.119), and the overall explanatory power of the model is high (R^2 of 0.9306). The –8.25% mean percent error indicates that the model somewhat overestimates CPUE values observed in the Bristol Bay red king crab fishery from 1991 through 1997. Out of 235 final aggregated observations, 50.6% are overestimated and 49.4% underestimated by the catch – soak time model. The model averages a 19.74% mean absolute percent error in fitting observed CPUE values (i.e., the model errs by an average of 1.59 crabs per pot lift). It should be noted that this mean absolute percent error is inflated by three prediction errors that are very small in nominal terms but very large in percentage terms, a consequence of some pots catching very few crabs. These three observations account for 5% of the 19.74% error and represent only 319 pot lifts (0.1% of all observed pot lifts). The three observations were omitted and the model was reestimated. Similar parameter estimates and P and R^2 values were obtained. Other goodness-of-fit measures improved to 14.73 for mean absolute percent error and –3.34 for mean percent error. In the end, we decided to retain all usable data because we had no way of knowing whether these three observations were true outliers or simply reflective of setting gear on no crab.

Graphical representation of model 3.2 is presented in Fig. 1. Year-specific CPUE – soak time curves depict predicted catch from pots dropped on the first day of the season (DT = 0). The slopes of CPUE – soak time curves attenuate as drop time progresses.

Discussion

CPUE – soak time relationships for Bristol Bay red king crab were modeled as elementary von Bertalanffy type equations nested within more general specifications that capture

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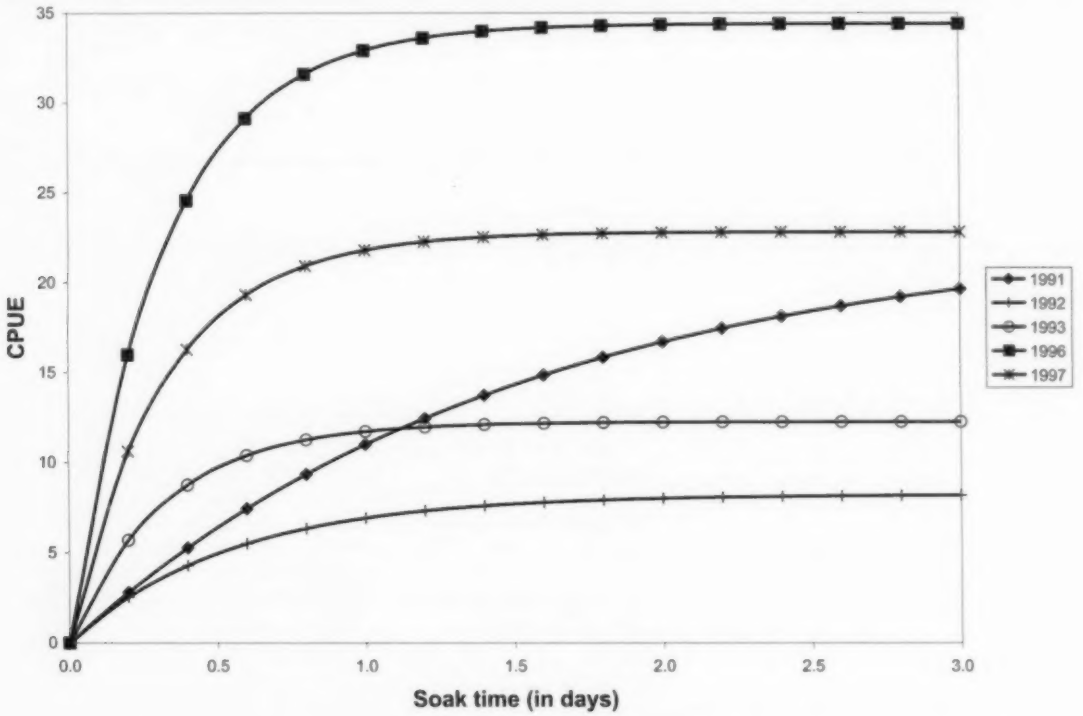
Fig. 1. CPUE – soak time relationships: model 3.2 estimated at DT = 0.



aggregate intra- and inter-seasonal variations. Data pooling and recursive hypothesis testing were utilized to address limited data variability and other commercial fisheries data deficiencies. The estimated model suggests that at the beginning of the season, fishers could potentially catch, on average, 22.81 crabs per pot in 1991 and 1997, 8.21 crabs per pot in 1992, 12.26 crabs per pot in 1993, and a high of 34.39 crabs per pot in 1996. That high-CPUE year was the year in which the smallest fleet (196 vessels, down from the 5-year average of 265 vessels) harvested 68% more crabs than expected in just a 4-day season. A total of 3810 tons of crabs was harvested, while the GHL was set at only 2268 tons. The effect of drop time on the maximum catch was more important in 1996 compared with the other years. The maximum potential catch per pot decreased by 20% after 1 day in 1996 and by 7% in 1991–1993 and 1997, suggesting that stock aggregations may have been more dense in 1996. A dense stock of crab increases catchability but contributes to a more rapid decline in the remaining stock available to harvest.

The basic CPUE – soak time relationship changed during the 1991–1997 period. In 1991, a 1-day soak achieved 66% of the catch that would result from soaking a pot for 2 days. The rate at which the pot fished toward its 2-day potential increased in subsequent years. In 1992, pots achieved 87% of a 2-day soak catch, after a single day. That rate increased to 96% in 1993–1997. (Rates at which pots fill were derived from model 3.2 using calculated CPUE values at different soak times.) These observations together suggest that a structural change might have occurred in the fishery, although inadequate data prevent isolating the components of change. Nevertheless, it lends insight into a change in fishing behavior. It is rational for individual crabbers to shorten soak time in order to increase their seasonal catch. Of course, when this behavior is extended across the entire fleet, individual shares remain relatively constant but the season shortens.

Crabbers adjust fishing strategies not only in response to biological and environmental factors, the object of the experiment-based literature, but also in response to policy-induced considerations. During the 1991–1997 period, pot limits were introduced and then halved in 1997 in an attempt to slow the fishery and better facilitate in-season management/fishery closure. Yet, the data indicate that average soak time shortened from as many as 2 days in 1991 to less than 1 day when the most restrictive pot limit was implemented. While there was an incentive to reduce soak time from 2 days to 1 day, even in 1991 when two 1-day soaks would have yielded 32% more crab as one 2-day soak, this did not occur. It would appear that crabbers may have learned to shorten soak times, possibly because they were restricted in the amount of available gear beginning in 1992. It was not until 1997, however, that the pot limit policy left crabbers with so little gear that they had to pull all pots every day or remain idle to maintain longer soak times. It follows that if the observed shift from a 2-day soak to a 1-day soak was due primarily to the pot limit policy, the policy was counterproductive. Behavioral response from the crabbers may have defeated the policy intent and exacerbated the in-season management problem. Ironically, the pot limit policy may

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have educated fishers that short soaks are effective ways to maximize seasonal catch during short-season, open-access fishing derbies like that of Bristol Bay red king crab.

Such a policy inference should be regarded as tentative because the empirical analysis was based on after-season dockside surveys that lacked scientific experimental design and, in general, were not taken from contemporaneous skipper logbook records. Nevertheless, the fact that after-season dockside surveys continue to be collected suggests that the managers believe that the data reflect general fishery performance. If one accepts the veracity of the survey data as "representative" of fleet behavior, then the results of our analysis may be presumed to approximate the underlying CPUE – soak time relationship for the fishery. At the very least, additional research into the impact of pot limits on season length seems warranted.

Lack of either daily observations on fleet-wide activity or a scientifically designed sample of daily fleet activity complicated the statistical analysis and ultimately required the use of indicator variables to capture only the aggregate presence of inter- and intra-seasonal CPUE – soak time variation. Specific causal mechanisms identified in the experiment-based literature and by industry as factors contributing to inter- or intra-seasonal variation (e.g., initial and daily biomass or general environmental conditions like water temperature, weather, or tides) could not be investigated because they require more data-intensive model specifications. Accordingly, while the results of this model may be useful as a descriptor of past behavioral responses to conditions affecting the fishery, usefulness for forecasting future fishery performance and for enhancing in-season management is likely quite limited.

Richer, more data-intensive specifications conceivably could have been estimated had real-time daily fishery performance observations been collected from the entire fleet. Such data collection capability now is both technologically feasible and relatively inexpensive. Complete enumeration of daily vessel-by-vessel activity during 1991–1997 would have yielded approximately 9400 daily vessel observations compared with the 235 aggregated observations used in this study. This 40-fold increase in observations should capture much greater variation in vessel performance and accommodate added model parameterization that facilitates forecasting. The potential to both forecast and hindcast fishery performance should be a compelling impetus for fishery managers and industry alike to collect real-time data that could assist in-season management of this very valuable but vulnerable fishery.

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The influence of smallmouth bass (*Micropterus dolomieu*) predation and habitat complexity on the structure of littoral zone fish assemblages

Pamela S.D. MacRae and Donald A. Jackson

Abstract: Fish assemblages in small lakes (≤50 ha) in central Ontario were characterized to determine the impact of smallmouth bass (*Micropterus dolomieu*) predation and habitat complexity on the structure of littoral zone fish assemblages. Data were collected employing minnow traps and visual assessment. Although species richness did not differ between lakes with and without smallmouth bass, species composition and relative abundance did differ. We identified two distinct fish assemblage types: one characterized by small-bodied species, mainly cyprinids, and a second by large-bodied centrarchid species, e.g., smallmouth bass. Smallmouth bass appear to reduce abundance, alter habitat use, and extirpate many small-bodied species such as brook stickleback (*Culaea inconstans*), fathead minnow (*Pimephales promelas*), pearl dace (*Margariscus margarita*), and *Phoxinus* spp.

Résumé : La caractérisation des peuplements de poissons du littoral de petits lacs (≤50 ha) du centre de l'Ontario a permis d'évaluer l'impact de la prédation de l'Achigan à petite bouche (*Micropterus dolomieu*) et de la complexité de l'habitat sur la structure des communautés. Les données consistaient en des récoltes faites à la nasse et des estimations visuelles. La composition spécifique des peuplements de poissons et l'abondance relative des espèces différaient entre les lacs où l'achigan était présent et ceux où il était absent, mais pas la richesse en espèces. Il a été possible de distinguer deux types de peuplements, l'un composé de poissons à corps élancé, en majorité des cyprinidés, et l'autre dominé par des poissons centrarchidés à corps massif, e.g., l'achigan. La présence de l'achigan semble donc réduire l'abondance des poissons, modifier l'utilisation de l'habitat et éliminer plusieurs des poissons à corps élancé, tels que l'Épinoche à cinq épines (*Culaea inconstans*), le Tête-de-boule (*Pimephales promelas*), le Mulet perlé (*Margariscus margarita*) et les *Phoxinus* spp.

[Traduit par la Rédaction]

Introduction

Human-induced environmental disturbances have led to the alteration of species distributions around the world. Concern about the introduction of nonnative species into aquatic ecosystems has led to a growing concern regarding the loss of native fish biodiversity (Chapleau et al. 1997). Most fish introductions, whether intentional or unintentional, have negative effects on native fishes and other taxa through predation, competition, hybridization, and introduction of diseases (Allendorf 1991) and possibly habitat alteration, trophic alterations, and gene pool deterioration (Crossman 1991).

Temperate lakes contain fish communities whose composition and richness are shaped by a number of biotic and abiotic factors, and piscivory appears to be one of the major factors contributing to community structure (Tonn and Magnuson 1982). Piscivores alter small-fish assemblage composition in small boreal lakes by excluding predation-intolerant species (Harvey 1981; Tonn and Magnuson 1982) among other effects. Because piscivores are size-selective and gape-limited (Tonn and Paszkowski 1986; Post and Evans 1989), small fishes are generally more vulnerable to predation than large fish (Tonn et al. 1992), although it may be a combination of size and morphology that determines risk to predation (He and Kitchell 1990. These small-bodied species could potentially be eliminated from a lake if local predators are able to eat all size classes (Tonn et al. 1992). Therefore, small-bodied species are often reduced in richness (Jackson 1988) or absent from small lakes containing littoral piscivores (Harvey 1981; Tonn and Magnuson 1982; Jackson et al. 1992). Vulnerable species vary by lake but studies have shown certain small-bodied species to be particularly susceptible to piscivorous fish predation (Jackson 1988; Naud and Magnan 1988; He and Wright 1992). Recruitment of larger-bodied species may be regulated by piscivores but these species would not likely be eliminated from lakes if adults attained a size large enough to escape predation (Tonn et al. 1992).

It is generally accepted that complex habitats are safer for prey than more open habitats (Crowder and Cooper 1982). Complex habitats provide greater amounts of refuge for fish, refuge being defined as a physical location where the prey can live or temporarily hide to live (McNair 1986). However, more open habitats can be more profitable for resources than

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Table 1. Location and surface area of Ontario lakes and presence or absence of smallmouth bass.

Lake	Location	Area (ha)	Smallmouth bass
Grindstone (GR)	45°11′N, 78°52′W	32	Present
Heron (HE)*	45°27′N, 78°49′W	24	Present
Kearney (KE)*	45°35′N, 78°27′W	32	Present
Little Wren (LWR)	45°11′N, 78°51′W	16	Present
Louie (LO)	45°23′N, 78°44′W	31	Present
Plastic (PL)	45°11′N, 78°50′W	33	Present
Wren (WR)	45°11′N, 78°42′W	50	Present
Bluff (BL)	45°35′N, 78°20′W	8	Absent
Jake (JK)	45°34′N, 78°33′W	6	Absent
Marmot (MR)	45°41′N, 78°24′W	3	Absent
Poorhouse (PRH)	45°22′N, 78°45′W	30	Absent
Scott (SC)	45°29′N, 78°43′W	28	Absent
Sproule (SP)*	45°36′N, 78°23′W	43	Absent
Sunday (SU)*	45°36′N, 78°21′W	46	Absent

Note: Sampling dates include cycle 1 (May 15–18), cycle 2 (May 21–26), cycle 3 (June 7–18), cycle 4 (June 28 – July 10), cycle 5 (summer survey, July 26 – August 21), and cycle 6 (September 9–13).

*Sampled repeatedly throughout the course of the summer.

complex habitats (Werner et al. 1983). The trade-off between resource availability and predation risk determines the habitat use patterns of fish (Mittlebach 1981; Werner et al. 1981; Crowder and Cooper 1982), and species have been shown to reduce the risk of predation by finding refuge at the expense of foraging efficiency (Phelan and Baker 1992).

Piscivorous fishes have the ability to influence the distribution of their prey, which may alter the feeding habits of the prey by constraining their feeding space or time, preventing their colonization of habitats or causing them to emigrate, which all may ultimately reduce their numbers (Power et al. 1985; Jackson et al. 2001). Tonn and Magnuson (1982) describe the distinction of lakes dominated by large piscivores verses Umbra-cyprinid species, where the small bodied species were either rare or absent from lakes with large piscivores. Many fish populations have been eliminated, in large part, because of predation (Zaret and Paine 1973).

Knowing the importance of complex habitat as refuge, we recognize the need to protect these fish habitats. However, with the continued development of lakes, there is an increasing trend to remove important fish habitat components such as woody debris and macrophytes. Lake development is coupled with the alteration of shoreline fish habitat. Developed lakes, those lakes influenced by human land use, have less woody habitat than undeveloped lakes (Christensen et al. 1996). Bryan and Scarnecchia (1992) compared developed and undeveloped lakes and found fish richness and abundance to be greater in undeveloped lakes, which contained a greater proportion of complex vegetation structure. Lakes undergoing shoreline development are the same lakes with high fishing pressure, making them particularly susceptible to sport fish introductions due to the increase in accessibility of the lake (Jackson et al. 2001). As well, piscivores have been demonstrated to prosper in some developed habitats. Bryan and Scarnecchia (1992) discovered that smallmouth bass (*Micropterus dolomieu*) were more abundant in developed lakes compared with undeveloped lakes. Yet species, largely game species, are often introduced into lakes with little regard to their impact aside from their recreational opportunities.

In this study, we investigated the long-term impact of piscivorous fish on the littoral fish community structure of small temperate lakes. There is a long history of smallmouth bass introductions into Ontario lakes, although they are not well documented. Smallmouth bass were introduced into lakes in Algonquin Provincial Park, Ontario, beginning in the early 1900s (Christie 1957) and have been introduced into other lakes to supplement the sport fishery for native salmonids (Ridgway et al. 1991). From these initial introductions, natural colonization of watersheds has occurred. As a result of the continued introduction of smallmouth bass, concern is being raised regarding the potential impact that they may have on indigenous species. In the absence of pre-introduction fish community data, lakes lacking piscivores can be used to assess the impact of smallmouth bass introductions on native fish communities (Chapleau et al. 1997).

We sought to quantify the long-term impact of smallmouth bass on fish community structure by asking the following questions. Does total species richness and abundance of littoral fishes differ between lakes with and without smallmouth bass? What effect do smallmouth bass have on the use of complex and simple habitats by species vulnerable to smallmouth bass predation? Does a difference exist in the size of species vulnerable to predation between complex habitats offering refuge and those habitats of simple, more open areas in lakes with and without smallmouth bass?

Methods

Study design

This project comprised two studies. One was an intensive examination of fish assemblages in four lakes during May to September using visual assessment and trapping techniques to identify and count fish. The second, a study of 14 lakes sampled during maximum summer water temperatures, was a broader study with less intensive sampling but a larger number of lakes. Lakes chosen for this study were all ≤50 ha (mean surface area of 31 ha for lakes with smallmouth bass and 26.3 ha for lakes without smallmouth bass); secluded lakes were preferred to reduce tampering with sampling gear and other sources of disturbance by the public. Lakes chosen were divided into two groups: lakes with smallmouth bass, an introduced littoral piscivorous predator, and lakes without smallmouth bass. The dates of smallmouth bass introduction or colonization are unknown and varied among lakes.

Four lakes were sampled for fish during May through September 1998. Each lake was sampled on six different occasions, referred to as the sampling cycles (Table 1). This portion of the project was designated the "base study." In the larger study, 14 lakes (Table 1) were sampled for fish during mid-July through mid-August (Table 2) and designated the "summer survey." These lakes were sampled only once to increase sample size. Base study results revealed that the combination of trap and visual data for 1 day of sampling is representative of a littoral fish community (MacRae 1999). In the base study, an initial shoreline cruise was done to record relative habitat complexity of the littoral zone. Six sites were chosen per lake, three complex habitats and three simple habitats to maximize the habitat contrast. The design was to contrast habitat types with high complexity offering fish refuge versus low-complexity or simple sites offering little to no cover available to fish in order to

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Table 2. Presence (1) – absence (empty cell) data from the combined sampling using minnow traps and visual sampling from Ontario lakes.

	Lakes with smallmouth bass							Lakes without smallmouth bass						
Species	GR	LO	WR	PL	LWR	HE	KE	SU	SP	PRH	JK	BL	MR	SC
Rock bass (RB)		1	1	1	1									
Largemouth bass (LMB)			1		1									
Smallmouth bass (SMB)	1	1	1	*	1	1	1							
Yellow perch (YP)	1	1	1	1	1	1	1	1	1					
Common shiner (CS)			1		1	1	1	1	1					
Bluntnose minnow (BNM)		1			1	1	1			1				
Pumpkinseed (PS)	1	1	1	1	1	1	1	1		1	1			
Brown bullhead (BBH)					1			1						
Blacknose shiner (BNS)						1	1	1	1	1				
Creek chub (CC)		1		1	1	1	1	1	1	1	1			
Pearl dace (PD)								1	1					
Iowa darter (ID)									1					
White sucker (WS)						1	1		1			1		
Golden shiner (GS)			1		1		1		1	1	1	1	1	
Phoxinus spp. (PH)								1	1	1	1	1	1	1
Brook stickleback (BRS)										1	1	1		1
Fathead minnow (FHM)										1	1	1	1	1
Brook trout		*				*	*	*	*	*	*		*	*
Lake trout		*	*		*	*	*	*						*
Splake								*	*					*
Burbot			*		*	*	*	*						

Note: Lakes and species are ordered as they appear along the first axis in the correspondence analysis (Fig. 1) to show the pattern in species assemblages. See Table 1 for lake name abbreviations.

*Piscivorous species previously found in the lake (e.g., Jackson 1988) but not captured during this study.

identify whether there are differences in habitat use by fish in the presence and absence of smallmouth bass. Complex habitats had high quantities of coarse, large woody material and (or) vegetation. Simple habitats were composed mainly of sand, gravel, rocks, and boulders, with little to no wood or vegetation. Although the degree of complexity within either category may vary across the lakes, the within-lake contrast is the relevant level of comparison. Sites were selected as evenly distributed around the lake as possible within the habitat designations. To maintain independence of sampling areas, areas were at least 60 m from one another. Visual sampling and trapping were conducted within each of the chosen sites. This methodology resulted in a stratified, randomized design (McInerny and Degan 1993; Whittier and Hughes 1998). Through the summer survey, all sites sampled visually and by trapping were randomly chosen. Visual sampling was completed throughout the entire shoreline in small lakes (≤6 ha).

Sampling protocol

All sites within lakes were swum using snorkeling gear to record presence–absence data of fish species. A total of ten 5-m transects were set 1–1.5 m from shore at a depth of 1–2.5 m at each site selected to ensure that species that may not be caught in minnow traps due to species selectivity and bias would be included in the presence–absence data. Lakes were then sampled with 18 baited minnow traps to assess the relative abundance of the fish assemblage. These were commercially available Gee minnow traps with an opening diameter of 2–3 cm. Traps were baited with a dry cereal based dog kibble and were set near the shoreline at depths ranging from 0.5 to 2 m. Traps were set over night and collected in the morning, after about 16 h of sampling. Fish collected were counted and identified to species (Table 2). Minnow traps were shown to provide a good estimate of the littoral fish communities based on comparisons with other sampling protocols and known community composition (MacRae 1999). Subsamples of a maximum of 25 individuals of each species were chosen as encountered and total length was measured. Only small-bodied species, e.g., cyprinids, and the young of larger species were collected in minnow traps due to size selection of traps. However, it is these small species, as well as the young-of the-year from other larger-bodied species, that are most vulnerable to smallmouth bass predation.

Statistical analysis

Relative abundance estimates of fish data were derived from mean catch per unit effort (XCPUE) of fish collected from minnow traps. XCPUE estimates of species relative abundance were compared for each species between lakes with and without smallmouth bass, and length of fish and mean total length per species were compared between habitat types. Comparisons employed a t test assuming unequal variance. A one-way test was employed for vulnerable species because an assumption of distribution was apparent, while all other species were compared using a two-way distribution test. Combined results from individual species comparisons were tested using the combined probabilities test (Sokal and Rohlf 1995) and a binomial test of trends in species abundance between habitats.

Correspondence analysis (e.g., Jackson and Harvey 1989) was used to summarize the underlying composition of fish communities and lake similarity and to graphically demonstrate the separation of species–lake relationships characterized in this study. Species and lakes positioned in similar ordination space represent species–lake associations that occur together more frequently than would be expected if species were randomly distributed across the lakes. Correspondence analysis results are presented for the summer survey using presence–absence data and relative abundance estimates (XCPUE). Species caught or observed only once in a single lake were excluded.

Results

Eighteen species were sampled from these lakes during the base study and summer survey combined and include

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Table 3. Comparison of fish abundance estimates (mean catch per unit effort (XCPUE)) between lakes with and without smallmouth bass in the summer survey.

	XCPUE				
Species	Lakes with smallmouth bass	Lakes without smallmouth bass	Difference	Probability	Trend
Blacknose shiner	0.01	0.04	–0.03	0.08	Negative
Bluntnose minnow	0.49	3.73	–3.33	0.20	Negative
Brook stickleback	0	0.31	–0.31	0.08	Negative
Fathead minnow	0	38.15	–38.15	0.01	Negative
Pearl dace	0	0.08	–0.08	0.15	Negative
Phoxinus spp.	0	38.64	–38.64	0.03	Negative
Brown bullhead	0	0.01	–0.01	0.36	Negative
Common shiner	0.18	0.08	0.10	0.59	Positive
Creek chub	1.10	1.20	–0.10	0.91	Negative
Golden shiner	0.08	1.59	–1.50	0.11	Negative
Largemouth bass	0.01	0	0.01	0.36	Positive
Pumpkinseed	4.76	1.02	3.73	0.05	Positive
Rock bass	0.82	0	0.82	0.09	Positive
Smallmouth bass	0.06	0	0.06	0.07	Positive
White sucker	0	0.14	–0.14	0.29	Negative
Yellow perch	0.53	0.28	0.25	0.38	Positive

Note: "Difference" category is the difference between the mean abundance values per minnow trap for the lakes with smallmouth bass minus the mean abundance values for the lakes without smallmouth bass. The probability value is for the associated *t* test.

those species caught in minnow traps or observed in visual samples. Brook trout (*Salvelinus fontinalis*), lake trout (*Salvelinus namaycush*), and splake (*S. fontinalis* × *S. namaycush*) have been captured in these lakes in previous studies using other types of sampling gear (Table 2). These salmonids inhabit cool or cold water during the summer months and would not be expected in the warm littoral zone due to high temperature. Two species occurred in only one lake; burbot (*Lota lota*) and Iowa darter (*Etheostoma exile*) were found in Sproule Lake. Largemouth bass (*Micropterus salmoides*) was found in only two lakes, Wren Lake and Little Wren Lake, that are connected by a short watercourse. Only young-of-the-year were found in both lakes, and largemouth bass were not found during previous surveys (Jackson 1988) but were known to be in nearby lakes. They may have colonized or been introduced into these two lakes since that sampling.

In the base study, species richness did not differ between lakes with and without smallmouth bass. However, reductions in abundance or the absence of small-bodied species, including blacknose shiner (*Notropis heterolepis*), bluntnose minnow (*Pimephales notatus*), pearl dace (*Margariscus margarita*), and *Phoxinus* spp. (northern redbelly dace (*Phoxinus eos*) and (or) finescale dace (*Phoxinus neogaeus*)), were observed in lakes with smallmouth bass. Abundance estimates for fish between base study lakes or habitat types were not compared statistically due to low sample size (four lakes). From the 14 lakes sampled in July through August, no significant difference in species richness between lakes with and without smallmouth bass was observed (Mann–Whitney $U = 22$, $p = 0.75$). However, species most vulnerable to predation were absent from lakes with smallmouth bass. Species absent or lower in abundance in lakes with smallmouth bass include the fathead minnow (*Pimephales promelas*) ($p = 0.01$), *Phoxinus* spp. ($p = 0.03$), brook stickleback (*Culaea inconstans*) ($p = 0.08$), and blacknose shiner ($p = 0.09$) (Table 3). An overall significance test showed a greater abundance of vulnerable species in lakes without smallmouth bass ($\chi^2_{12} = 33.31$, $p = 0.001$; binomial distribution test (BNMDT), $p = 0.01$) (Table 4*a*) relative to those lakes with smallmouth bass. A comparison of all other species in lakes showed mostly increased but nonsignificant trends in species abundance in smallmouth bass lakes compared with those lakes without smallmouth bass (Table 3). Overall significance tests indicated no difference in these species abundances between lakes with and without smallmouth bass ($\chi^2_{20} = 30.47$, $p = 0.63$; BNMDT, $p = 0.20$) (Table 4*a*). Although species richness did not differ between lakes with and without smallmouth bass, their species compositions were distinct. Lakes with smallmouth bass contained 2.3 fewer small-bodied species, on average, than lakes without smallmouth bass.

Results did not reveal significant differences in species abundance between habitat types; however, comparisons of abundance estimates between habitat types showed a greater abundance of vulnerable species in complex over simple habitats where subject to smallmouth bass predation. While no significant difference in abundance of fish between habitats was found ($\chi^2_4 = 5.70$, $p = 0.22$; BNMDT, $p = 0.25$; nonsignificance is due to low sample size (n = 2)) (Table 4*b*), low sample size and statistical power is due to the fact that four of the six vulnerable species, brook stickleback, fathead minnow, pearl dace, and *Phoxinus* spp., were absent from all summer survey lakes containing smallmouth bass. This situation results from these species often being unable to persist in lakes with smallmouth bass; however, not all lakes without bass contain all of these small-bodied species. Most other species also showed a trend of greater abundance in complex over simple habitats in lakes with smallmouth bass (Table 5). The overall test of significance showed a preference of complex habitats ($\chi^2_{12} = 26.05$, $p = 0.053$; BNMDT, $p = 0.03$), indicating a greater abundance of

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Table 4. Overall tests of significance (χ^2 and binomial test) for differences (*a*) in relative abundance between lakes with and without smallmouth bass, (*b*) in relative abundance between habitats for both vulnerable species and all other species, (*c*) in total length of species between habitats in the base lakes, and (*d*) in total length of species between habitats in lakes with and without smallmouth bass.

	Observed χ^2 value	Critical χ^2 value	Associated probability	Binomial probability
(*a*) Relative abundance in lakes with smallmouth bass versus lakes without smallmouth bass				
Vulnerable species	33.31		0.001	0.01
Other species	30.47		0.63	0.21
(*b*) Relative abundance between habitats in lakes with smallmouth bass versus lakes without smallmouth bass				
Vulnerable species				
Bass: complex versus simple habitat	5.70		0.22	0.25
No bass: complex versus simple habitat	20.95		0.05	0.09
Other species				
Bass: complex versus simple habitat	26.05		0.05	0.03
No bass: complex versus simple habitat	16.31		0.29	0.27
(*c*) Differences in length of fish between simple and complex habitats in base lakes				
Heron Lake	6.25		0.9	0.31
Kearney Lake	12.01		0.44	0.23
Sunday Lake	22.97		0.29	0.21
Sproule Lake	7.87		0.89	0.27
(*d*) Differences in length of fish between lakes with smallmouth bass versus lakes without smallmouth bass in the summer survey				
Bass	11.57	21.02	0.48	0.09
No bass	5.81	28.86	0.99	0.16

nonvulnerable species in complex habitats relative to simple habitats in lakes with smallmouth bass.

A comparison of abundance estimates between complex and simple habitats in lakes without smallmouth bass showed reduced abundance of vulnerable species in the complex habitats (Table 5). Combined probability and binomial distribution tests reveal a greater abundance of vulnerable species in simple habitats ($\chi^2_{18} = 20.95$, $p = 0.051$; BNMDT, $p = 0.09$). All other species showed no trend in habitat preference in lakes when smallmouth bass were not present ($\chi^2_{20} = 16.31$, $p = 0.295$; BNMDT, $p = 0.27$).

Length data

Minnow traps are size selective given their limited size of the opening; however, those species considered vulnerable to smallmouth bass predation would not be limited by the trap's opening. No clear trends existed in the size of these fish in the two habitats of the lakes with and without smallmouth bass in the base study. Overall, significance tests revealed that total length of fish, mean total length per species across species, did not differ between habitats in lakes with and without smallmouth bass (Heron Lake: $\chi^2_{12} = 6.25$, $p = 0.90$; BNMDT, $p = 0.31$; Kearney Lake: $\chi^2_{12} = 12.01$, $p = 0.44$; BNMDT, $p = 0.23$; Sproule Lake: $\chi^2_{14} = 7.87$, $p = 0.89$; BNMDT, $p = 0.27$; Sunday Lake: $\chi^2_{20} = 22.97$, $p = 0.29$; BNMDT, $p = 0.21$) (Table 4*c*). The summer survey revealed no trend for total length of species in the two habitats in lakes subject to smallmouth bass predation ($\chi^2_{12} = 11.57$, $p = 0.48$; BNMDT, $p = 0.09$) (Table 4*d*). There was no significant difference in total length of all species between habitats in lakes without smallmouth bass ($\chi^2_{22} = 5.81$, $p = 0.99$; BNMDT, $p = 0.16$).

Species–lake relationships

Presence–absence data

Correspondence analysis revealed a separation of lakes with and without smallmouth bass along the first axis (Fig. 1). Base lakes, Sproule Lake and Sunday Lake, were together and were separated from other lakes along the first and second axis by rare species (brown bullhead (*Ameiurus nebulosus*), Iowa darter, and pearl dace). Lakes with smallmouth bass were positioned at coordinates similar to the larger centrarchid species, whereas lakes without smallmouth bass were closely associated with small-bodied species, mainly cyprinids and brook stickleback (Fig. 1). Species close to the origin (e.g., common shiner (*Luxilus cornutus*), creek chub (*Semotilus atromaculatus*), pumpkinseed (*Lepomis gibbosus*), and yellow perch (*Perca flavescens*)) were common species and (or) were found in most lakes regardless of the presence or absence of smallmouth bass. Species positioned at the far left end of axis 1 (e.g., brook stickleback, fathead minnow, *Phoxinus* spp.) were found frequently in the same lakes. This group of species was positioned separately from the group containing smallmouth bass, largemouth bass, and rock bass (*Ambloplites rupestris*), indicating that these two groups of species were not found in the same lakes. Blacknose shiner and bluntnose minnow were positioned between lakes with and without smallmouth bass and showed little affinity towards being in lakes either with or without smallmouth bass.

Relative abundance data (XCPUE)

Correspondence analysis employing XCPUE data showed results similar to those of the presence–absence plot. Lakes with and without smallmouth bass were separated along the first axis (Fig. 2). Sproule Lake and Sunday Lake were positioned closer to Heron Lake and Kearney Lake, base lakes with smallmouth bass, due to high trap catches of blacknose shiner and common shiner in all of these lakes. Larger centrarchids were grouped together as were the lakes containing them, whereas small-bodied and rare species were associated with lakes without smallmouth bass. Four groups of species were identified. The first, mainly cyprinids, was associated with lakes without smallmouth bass and was

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Table 5. Comparison of fish relative abundance estimates (mean catch per unit effort (XCPUE)) between complex and simple habitats during the summer survey period.

Species	XCPUE		Difference	Associated probability	Trend
	Complex habitat	Simple habitat			
Lakes with smallmouth bass					
Blacknose shiner	0.01	0	0.01	0.18	Positive
Bluntnose minnow	0.38	0.31	0.07	0.32	Positive
Brook stickleback	0	0	0	na	
Fathead minnow	0	0	0	na	
Pearl dace	0	0	0	na	
Phoxinus spp.	0	0	0	na	
Brown bullhead	0	0	0	na	
Common shiner	0.26	0	0.26	0.33	Positive
Creek chub	1.61	0.18	1.43	0.19	Positive
Golden shiner	0.17	0	0.17	0.13	Positive
Largemouth bass	0	0.02	–0.02	0.36	Negative
Pumpkinseed	6.42	2.98	3.44	0.02	Positive
Rock bass	0.98	0.69	0.29	0.59	Positive
Smallmouth bass	0.07	0.06	0.01	0.62	Positive
White sucker	0	0	0	na	
Yellow perch	0.82	0.11	0.71	0.71	Positive
Lakes without smallmouth bass					
Blacknose shiner	0.03	0.05	–0.02	0.37	Negative
Bluntnose minnow	2.65	3.75	–1.10	0.18	Negative
Brook stickleback	0.19	0.27	–0.08	0.25	Negative
Fathead minnow	36.56	47.68	–11.12	0.25	Negative
Pearl dace	0.03	0.13	–0.09	0.22	Negative
Phoxinus spp.	43.58	33.70	9.88	0.03	Positive
Brown bullhead	0.02	0	0.02	0.36	Positive
Common shiner	0	0.02	–0.02	0.36	Negative
Creek chub	1.06	1.43	–0.37	0.35	Negative
Golden shiner	0.85	2.19	–1.35	0.28	Negative
Largemouth bass	0	0	0	na	
Pumpkinseed	1.52	0.53	0.99	0.34	Positive
Rock bass	0	0	0	na	
Smallmouth bass	0	0	0	na	
White sucker	0.08	0.21	–0.13	0.36	Negative
Yellow perch	0.32	0.24	0.09	0.19	Positive

Note: "Difference" category is the difference between the mean abundance values per minnow trap for the complex habitat minus the mean abundance values for the simple habitat. The probability value is for the associated *t* test (one-tailed test for the vulnerable species and two-tailed test for all other species). na, not applicable.

positioned at the left end of axis 1. The second group (e.g., creek chub and common shiner), a group of common species, was associated with the base lakes studied. The third group consisted of smallmouth bass and yellow perch, and the final group comprising other centrarchid species was associated with summer survey lakes containing smallmouth bass. Blacknose shiner and bluntnose minnow were positioned closer to the lakes without smallmouth bass compared with the presence–absence ordination (Fig. 1), indicating that although they were sometimes found with smallmouth bass, they were more abundant in lakes without smallmouth bass. Clearly, lakes characterized by centrarchids were separated from those with cyprinids and brook stickleback.

Discussion

An average of 5.4 fish species were found in the littoral zone and did not differ between lakes with and without piscivorous smallmouth bass. However, the fish community structure differed. Lakes with smallmouth bass had an average of 2.3 fewer small-bodied species compared with lakes without smallmouth bass. We identified two distinct fish assemblages, one characterized by a group of cyprinid species and brook stickleback and the other by large-bodied centrarchid species. The most apparent difference between the two assemblage types was the strong negative relationship between centrarchid assemblage and the brook stickleback – fathead minnow – *Phoxinus* spp. assemblage.

Piscivores alter small fish assemblage composition in small boreal lakes by excluding predation-intolerant species (Harvey 1981; Tonn and Magnuson 1982) through predation and (or) emigration of the small fish (He and Kitchell 1990). While our study showed clear separation of fish assemblage types between those lakes composed of large centrarchid species and those containing mainly small-bodied species, we also showed a trend in habitat use by small fishes in

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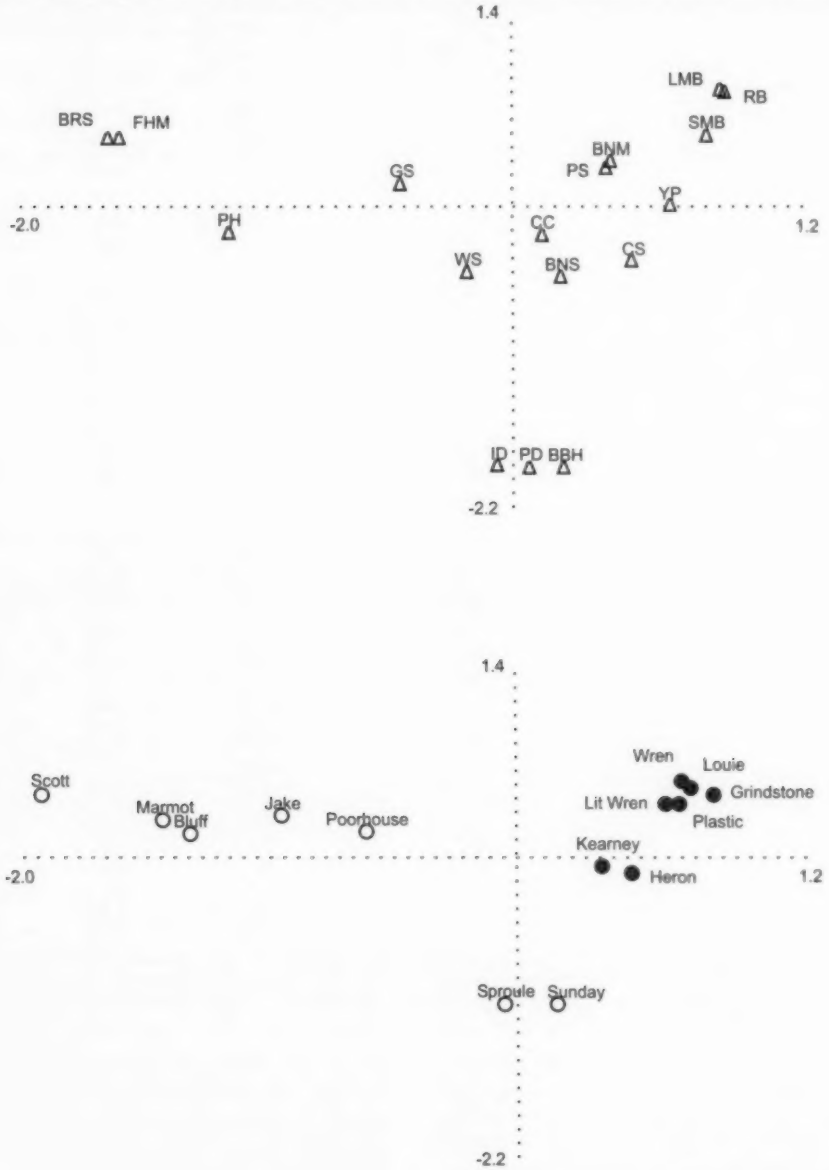
Fig. 1. Axes 1 and 2 from a correspondence analysis of species presence–absence for summer survey lakes. Solid circles identify lakes containing smallmouth bass, and open circles identify lakes without smallmouth bass. See Table 2 for species abbreviations.



these lakes where smallmouth bass were present. This distinction of lakes containing or lacking smallmouth bass is apparent, although other piscivorous species, i.e., salmonids, present in these lakes do not show such relationships with the small-bodied species. Summer thermal characteristics would separate the vulnerable species from the salmonids, thereby minimizing the predation effect during the periods of reproduction and greatest activity and growth for the small species. However, effects similar to the contrast between smallmouth bass and no smallmouth bass should be found with other strong littoral predators, e.g., northern pike (*Esox lucius*), but they were not present in these lakes. Results from our study indicate predation by smallmouth bass as being the most parsimonious explanation for the observed differences in richness of vulnerable species and overall differences in composition between lakes with and without smallmouth bass. Although these results are observational rather than manipulative, the evidence strongly supports predation as the primary factor causing the difference in the species composition in lakes. These results are comparable with those of other studies, including He and Kitchell (1990) and Chapleau et al. (1997).

We found that species vulnerable to predation in lakes without smallmouth bass were often more abundant relative

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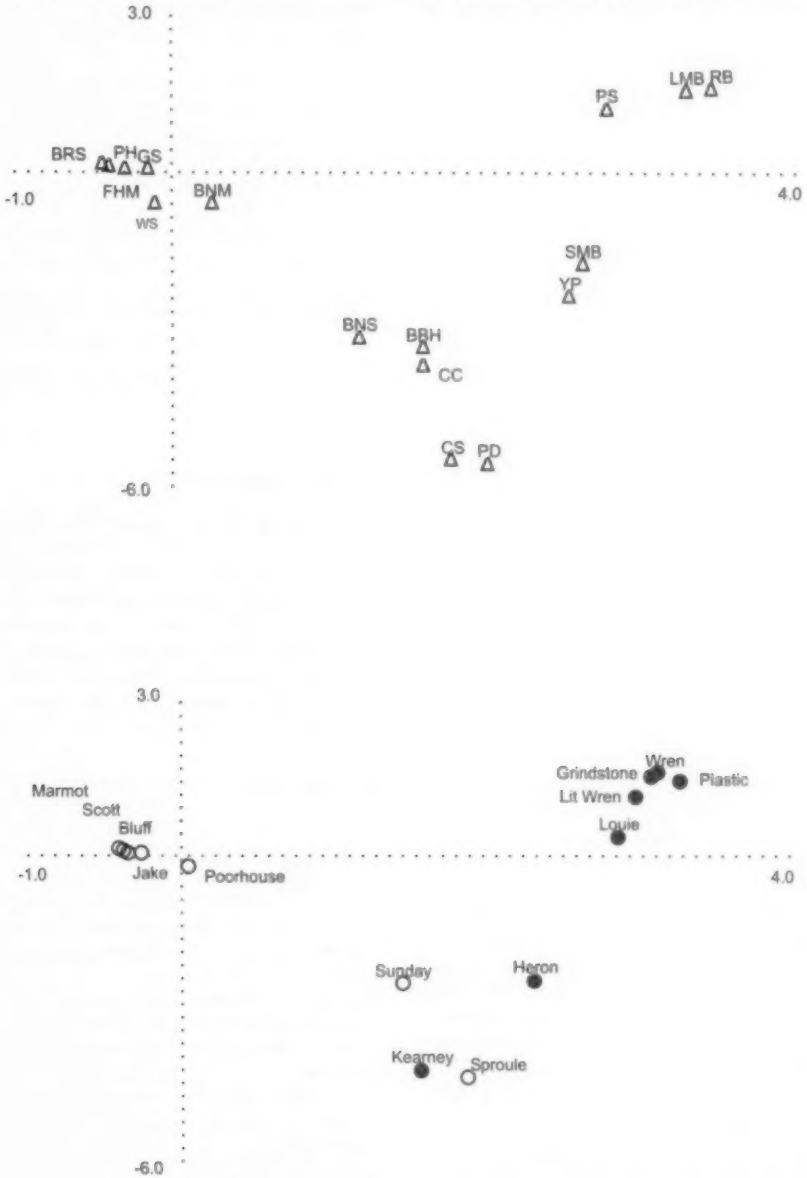
Fig. 2. Axes 1 and 2 from a correspondence analysis for summer survey lakes using XCPUE. Solid circles identify lakes containing smallmouth bass, and open circles identify lakes without smallmouth bass. See Table 2 for species abbreviations.



to populations in lakes with smallmouth bass, whereas most other species showed no clear trend in the abundance between these same lakes. Exclusion or reduction in abundance of species was limited to small-bodied species, whereas larger-bodied species (e.g., creek chub, yellow perch, and white sucker (*Catostomus commersoni*)) often coexisted with smallmouth bass populations. Blacknose shiner and bluntnose minnow, while often coexisting with smallmouth bass, were more abundant in lakes without smallmouth bass. Thus, predation by smallmouth bass has a greater influence on small-bodied species than on large-bodied species. This is consistent with He and Wright (1992), who observed a shift in community composition from small-bodied, soft-rayed species to large or deep-bodied species with spines following the introduction of northern pike.

Species subject to predation often seek refuge in structurally complex habitats (Crowder and Cooper 1982; McNair 1986). Although no statistically significant difference was found in habitat use of small-bodied species in lakes with smallmouth bass, a trend of greater abundance in complex habitat was observed. The fact that four of the six species considered vulnerable to smallmouth bass predation did not

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coexist with smallmouth bass limited the statistical power of this comparison. The complete absence of these species suggests that the lake conditions and habitat are insufficient to allow coexistence with smallmouth bass. In swimming all lakes, it was apparent that blacknose shiner and bluntnose minnow use the complex habitat to a greater degree than the simple habitat during the day. It is possible that these species take advantage of diel peaks in piscivore activity and feed in simple habitats during low light levels while the predators are less active (Naud and Magnan 1988). This would explain why traps were able to catch these small-bodied species when set overnight. However, these same species, when not subject to predation, do not confine themselves to complex habitats but occupy the entire littoral zone. Moreover, these same fish are often more abundant in simple rather than complex habitats. These results are consistent with other studies suggesting that high densities of vegetation and coarse and large woody structures offer refuge for small fish from predation (Crowder and Cooper 1982; Eklöv 1997). Fish will capitalize on resources throughout the lake where feasible, as restricting their foraging to only complex habitats would increase intra- and interspecific competition. This factor explains why we see vulnerable species more evenly dispersed between habitat types in lakes not influenced by smallmouth bass predation. Fish restricted to complex habitat may be unable to move between complex habitat patches to decrease intra- and inter-specific competition (Persson and Greenberg 1990). This implies that fish should use a greater range of habitats within the lakes; however, in lakes containing smallmouth bass, we see that the small-bodied species are reduced in their abundance, confined largely to the complex habitats, or simply not present.

From these results, we conclude that fish are choosing complex habitat to avoid predation because fish in lakes without smallmouth bass use both complex and simple habitat types. Presumably, those fish in simple habitats in lakes without smallmouth bass are taking advantage of additional food resources. In our small lakes, there is a negative relationship between the size of fish and structural complexity when fish are faced with predation. This indicates that smaller fish that are restricted to highly complex habitats would experience increased competition and less profitable foraging, whereas larger individuals of the same species are less prone to predation and can capitalize on other available resources in less complex habitats.

Our results highlight the importance of structural complexity as a habitat requirement of many littoral fishes, especially in the presence of littoral predators. Therefore, littoral habitat is particularly critical in lakes susceptible to fish introductions. Most species introductions occur in lakes with the most human activity (those containing cottages). These are the same lakes where cottage owners often remove both macrophytes and woody materials for a desired beach-like shoreline. Christensen et al. (1996) found that developed lakes with shoreline residences have lower densities of coarse woody debris compared with undeveloped lakes. Removal of such physical structures is known to have negative impacts on species composition and abundance of fish, benthos, and plankton communities in both freshwater and marine habitats (e.g., Poe et al. 1986; Everett and Ruiz 1993). Therefore, it is important to recognize the potential risk of local extinction and reduced species richness in developed lakes due to the combined stresses of predation pressure and lack of structured habitat.

Because the predation effect of smallmouth bass is so strong, presence–absence data provide valuable information pertaining to species–lake associations and are appropriate for most study objectives. However, relative abundance and length estimates may provide additional information on changes in species–lake relationships. Results of our study suggest that the introduction of smallmouth bass and other littoral piscivores to small lakes will lead to the marked reduction or elimination of those species most vulnerable to predation. Therefore, priorities should be identified for small lakes composed mainly of native, small-bodied species or diverse cyprinid communities and efforts be made to prevent the introduction of piscivores into these lakes and the alteration or homogenization of both lake habitat and fish communities.

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Validation of three back-calculation models by using multiple oxytetracycline marks formed in the otoliths and scales of bluegill × green sunfish hybrids

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Abstract: We assessed the accuracy of the Fraser–Lee, biological-intercept, and Weisberg back-calculation models to estimate growth from otoliths and scales of laboratory-reared juvenile bluegill × green sunfish hybrids (*Lepomis macrochirus* × *Lepomis cyanellus*). Hybrid sunfish were injected three times with oxytetracycline hydrochloride at 90-day intervals to mark bony structures, creating simulated annuli for model validation. Back-calculated lengths (BCLs) with otoliths were generally less accurate than scales for all three models. Errors ranged from –8.2 to 7.8% for the Fraser–Lee model, from –8.0 to 8.3% for the biological-intercept model, and from –6.5 to 14.3% for the Weisberg model. For all three models, there was no significant difference in BCLs using left or right otoliths, and BCLs using the Fraser–Lee and biological-intercept models were not significantly different from each other. In contrast with otoliths, all three models produced accurate BCLs from scales; errors ranged from –4.3 to 0.1%. For juvenile hybrid sunfish, we recommend using scales for back-calculation of growth. The Fraser–Lee (with zero intercept) and biological-intercept models produced the most accurate BCLs from otoliths. However, due to potential decoupling of otolith and body growth, caution should be exercised when estimating juvenile hybrid sunfish growth from otoliths.

Résumé : Nous avons évalué la précision des modèles de rétrocalcul de Fraser–Lee, de l'ordonnée à l'origine biologique et de Weisberg dans l'estimation de la croissance à partir des otolithes et des écailles chez des hybrides du Crapet arlequin et du Crapet vert (*Lepomis macrochirus* × *Lepomis cyanellus*) élevés en laboratoire. Les crapets hybrides ont été injectés à trois reprises avec de l'hydrochlorure d'oxytétracycline à intervalles de 90 jours afin de marquer les structures osseuses et ainsi créer des annulus simulés pour valider les modèles. Les longueurs obtenues par rétrocalcul à partir des otolithes étaient, en règle générale, moins précises que celles obtenues au moyen des écailles, et cela dans tous les modèles. Les erreurs variaient de –8,2 à 7,8% dans le modèle Fraser–Lee, de –8,0 à 8,3% dans le modèle de l'ordonnée à l'origine biologique et de –6,5 à 14,3% dans le modèle de Weisberg. Dans les trois modèles, les longueurs obtenues par rétrocalcul ne différaient pas, qu'on ait utilisé l'otolithe gauche ou le droit; les résultats obtenus par les modèles de Fraser–Lee et de l'ordonnée à l'origine biologique n'étaient pas significativement différents. Contrairement aux résultats obtenus à partir des otolithes, ceux provenant de l'étude des écailles étaient plus précis avec des erreurs allant de –4,3 à 0,1% dans les trois modèles. Nous recommandons donc d'utiliser les écailles pour le rétrocalcul de la croissance chez les jeunes crapets hybrides. Les modèles de Fraser–Lee (avec ordonnée à l'origine de 0) et de l'ordonnée à l'origine biologique ont fourni les meilleures estimations de BCL à partir des otolithes. Cependant, il est possible que la croissance des otolithes ne soit pas couplée à celle du corps; il faut donc faire preuve de prudence lorsqu'on veut estimer la croissance de jeunes crapets juvéniles par l'étude des otolithes.

[Traduit par la Rédaction]

Introduction

Back-calculation models are valuable because they provide growth data for fish populations from a single sampling event and have been used for over 80 years in fisheries management and research. In the field, fish length is measured and calcified structures (scales, otoliths, etc.) are collected for growth analyses. Subsequent age assessment and radial

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measurements to the annuli, along with the length of the fish at capture, are input into a back-calculation model that reconstructs the entire growth history of the individual fish for studies of population dynamics.

The economy and relative ease of obtaining growth histories with back-calculation have resulted in widespread use of this technique (DeVries and Frie 1996), yet few studies in the laboratory (Everhart 1948; Bradford and Geen 1987; Klumb et al. 1999*a*) have assessed model performance. Validation of back-calculation models provides fisheries managers and researchers confidence that observed growth differences within or among fish populations are real and not artifacts of invalid model assumptions or techniques. Francis (1990) listed three components needed to truly validate a back-calculation method: (*i*) the radius of yearly marks must remain constant from the time of formation (i.e., scale or otolith radii to respective annuli do not change once formed), (*ii*) the mark is a true annulus, formed on a yearly basis, and (*iii*) the formula used to back-calculate growth accurately describes the body–scale or body–otolith relationship. Requirements for conducting true validation are met only in field mark–recapture studies (Davies and Sloane 1986; Howells et al. 1995; Klumb et al. 1999*b*) or laboratory studies (Bradford and Geen 1987; Klumb et al. 1999*a*) where length measurements at the time of marking (the standard for comparison) are compared with back-calculated lengths (BCLs) from the same fish.

Casselman (1983) stated that the best evidence for validation requires data from fish marked with a fluorescent chemical or physical tag and then later recaptured, producing known increments of growth for comparison. Tetracycline antibiotics are incorporated into actively calcifying new bone, forming a yellow–gold mark visible under ultraviolet light (Weber and Ridgway 1962). Rapid deposition of tetracycline is required for accurate and precise marks, used as reference points in time, to form in the bones and scales of fish. Babaluk and Craig (1990) found that 100% of northern pike (*Esox lucius*) scales and otoliths were marked within 12 h. Within 24 h of injection, 100% of starry flounder (*Platichthys stellatus*) otoliths were successfully marked (Campana and Neilson 1982).

Bluegill (*Lepomis macrochirus*) and bluegill × green sunfish hybrid (*Lepomis macrochirus* × *Lepomis cyanellus*) scales have been successfully marked with oxytetracycline (OTC) by feeding, and these OTC marks were used for back-calculation model validation (Klumb et al. 1999*a*). No literature exists assessing the success of marking *Lepomis* sp. otoliths or scales by OTC injection. We present here the results of a laboratory experiment used to validate back-calculation growth models with OTC injection. The objectives of this study were to (*i*) assess marking success in otoliths and scales of bluegill × green sunfish hybrids raised in the laboratory and marked multiple times by injection with OTC, (*ii*) assess and validate the Fraser–Lee, biological-intercept (Campana 1990), and Weisberg (Weisberg 1986) back-calculation models, (*iii*) compare BCLs derived from three otolith radii with scales, (*iv*) compare otolith back-calculations using left and right otoliths from the same fish, and (*v*) determine the effect of known scale measurement error on BCLs.

Methods

Age-1 bluegill × green sunfish hybrids, hereafter referred to as hybrid sunfish, were injected three times with OTC at 90-day intervals (Kobayashi et al. 1964) to "simulate" annuli for back-calculation model validation; fish were generally of uniform total length (Table 1). Since fish were not individually tagged for identification (i.e., numbered tags), mean observed growth of all OTC-injected fish for each 90-day interval was compared with their mean BCL. The number and percentage of hybrid sunfish with multiple OTC marks in their otoliths and scales were assessed only for fish surviving the entire experiment.

Fish (N = 97) were injected intraperitoneally near the anus (Babaluk and Craig 1990) at a dosage of 75 mg OTC·kg fish^{-1} with Anchor OXY-TET 50 (oxytetracycline hydrochloride; Boehringer Ingelhein Animal Health Inc., St. Joseph, Mo.) sold at 50 mg OTC·mL^{-1}. If OTC appeared to seep from the injection wound, the fish was given a second injection. Immediate postinjection mortality was low; only one fish died within 1 day after injection. Control fish (N = 20), not injected with OTC, were raised in the same tank with the OTC-treated fish to ensure that no natural fluorescent marks in otoliths and scales occurred. The right pelvic fin and top lobe of the caudal fin were clipped to identify control fish.

To simulate "interannual" environmental variation, we attempted to vary growth rates between marking intervals by changing water temperature. Target temperatures for the three successive growth periods were 20, 15, and 25°C, respectively. Mean temperatures for each interval were within ±2°C of the targeted temperature (Table 1).

Two complete 90-day growth periods were completed with all fish, while a reduced number were used for the third growth period. A cracked submersible heater electrocuted 73 fish (55 experimental fish and 18 control fish); 38 experimental and two control fish survived and were used to complete the experiment. For the two groups of fish, BCLs were compared with the observed lengths at the first two OTC marks for the electrocuted fish and at all three OTC marks for fish surviving the entire experiment. Instantaneous growth rates for each 90-day interval were calculated using the formula in Ricker (1979).

Fish were raised in flow-through fiberglass raceways. Tanks received tap water at an average of 2 L·min^{-1}, providing about six water exchanges per day; current was negligible. Photoperiod was kept constant (17 h light : 7 h dark) and dissolved oxygen was monitored to ensure that levels remained above 5 mg·L^{-1}. Mean dissolved oxygen remained above 8 mg·L^{-1} throughout the experiment. Fish were fed daily at 2% of body weight with trout fry No. 4 granules (47% protein), 4.8-mm pellets (41% protein), or 3.2-mm pellets (40% protein) administered by 12- or 24-h automatic belt feeders. Ration was adjusted every 90 days after each OTC treatment or when excessive mortality occurred (i.e., total ration reduced proportional to mortality).

Sample collection and preparation

At the time of each OTC injection, all treatment and control fish were anesthetized with tricaine methanesulfonate (MS 222), total length was measured to the nearest millimetre (with depressed caudal fin), and weight was measured to the nearest 0.1 g. At the end of the experiment, otoliths (both sagittae) and scales (approximately 10–20) were collected. Left and right sagittae were analyzed separately. Otoliths for all species were wiped clean after removal and soaked in glycerin for 2 weeks to help them clear (Maceina and Betsill 1987). Scales were removed from the left side, below the lateral line, and anterior to the distal point of the pectoral fin when pressed against the body (Regier 1962).

Back-calculations were derived from three otolith radii: the anterior, dorsal, and posterior axes (Fig. 1). Otoliths were viewed

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Table 1. Mean total length of hybrid sunfish at the time of OTC marking, instantaneous growth rates, and water temperatures in the laboratory during the three 90-day experimental treatments used to validate back-calculation growth models.

OTC mark	*N*	Mean length ± 2 SE (mm)	Instantaneous growth rate (mm·day^{-1})	Mean water temperature ± 2 SE (°C)	Temperature range (°C)
1	97	119.9±2.6	0.002694	20±0.2	23–18
2*	101	152.8±3.4	0.001437	17±0.4	20–14
3†	37	173.9±4.8	0.000481	23±0.4	26–18

Note: Mean length ± 2 SE at the end of the experiment was 181.6 ± 5.6 mm.
*Four control fish were not identified and were included in the treatment group.
†Fifty-five experimental fish were electrocuted between OTC treatments 2 and 3.

whole with the sulcus side down. Sample sizes for the three axes differed slightly because no measurements were made along any axis that was chipped or broken or for fish without the expected number of OTC marks visible along a particular axis (Table 2). Measurements were made to the edge of the OTC mark proximal to the otolith core, and this point in time was assumed to correspond to body length measurements made before treatment (Campana and Neilson 1982).

The mean scale radius for each fish ($N = 3$) was used for back-calculation (Newman and Weisberg 1987). Scales were measured from the focus to the anteriolateral corner (anteriolateral axis) (Fig. 1). The edge of the OTC mark proximal to the scale focus was used as the point for validation of the back-calculation models and was assumed to have formed immediately after OTC treatment (Campana and Neilson 1982). For all fish, scale radii were measured from the scale focus to that point. No regenerated scales were used, and only scales from fish with the expected number of OTC marks were measured.

Because OTC marks typically create fluorescent bands that are two to three circuli wide in the scales of injected fish (Kobayashi et al. 1964; Bumguardner 1991), mark width was used to assess the effect of scale measurement error on BCLs. The widths of the OTC marks for 30 randomly selected fish were measured and the circuli in the OTC band were counted. Measurements were made with an occular micrometer at 10× magnification. To create a known measurement error, scale radii along the same anteriolateral axis were measured to the proximal and distal edges of the OTC mark for all fish. Known scale measurement error was calculated as the percentage of the total scale radius within the OTC fluorescent mark. Differences between BCLs from scale radii measured to the OTC mark inner and outer edges were used for model sensitivity analyses. Consistency of OTC mark width among treatments was tested with a one-way analysis of variance (ANOVA) and Bonferroni multiple-range test.

Detection of OTC marks and measurement techniques

The OTC marks in otoliths and scales were detected with an Olympus Vanox model AHBT compound microscope fitted with an AH2-RFL attachment for reflected and transmitted fluorescence. The excitation wavelength that provided the best fluorescence was the violet range (395–415 nm); Bumguardner (1991) used similar wavelengths. The factory-installed DM 455 dichroic mirror (455 nm), BP405 exciter filter (405 nm), and Y455 barrier filter (455 nm) were used without any supplementary barrier or exciter filters. The lamp source was a 200-W high-pressure mercury burner. Objective magnification was 2.5×.

High OTC mark intensity enabled otolith and scale radii to be measured with a digital image analysis system. Video images were created using an MTI series 68 mk II camera (Dage-MTI, Inc., Michigan City, Ind.) mounted on the microscope, and digital images were captured with a Targa+ framegrabber (Truevision, Inc., Indianapolis, Ind.). Images were processed and measured using the software Mocha™ v.1.2.10 (Jandel Scientific, San Rafael, Calif.). To view real-time images on a video monitor, an Olympus NFK 1.67× projection lens for the video camera was used for otolith images and an Olympus NFK 2.5× lens for scale images. Separate calibrations for otolith and scale images were done with a hemacytometer. Measurement error of the digital analysis system was 0.34% for otoliths and 0.48% for scales.

The OTC marks and the otolith core or scale focus required different techniques to maximize their contrast and resolution. Otoliths were immersed in glycerin during observation to improve contrast. A combination of brightfield and fluorescent microscopy was used to enhance contrast of the otolith core and scale focus. To properly locate the core or focus, a second image using brightfield illumination was made without moving the sample after collecting the image under fluorescent illumination. Annotation features in the software were then used to create an overlay on the brightfield image to locate the otolith core and scale focus, which was then overlain on the image with the visible OTC marks. A daylight/tungsten (blue filter), green filter, and neutral density filter were used during brightfield observation. Hybrid sunfish scales were soaked in glycerin for 2 days to clear and improve the contrast of the OTC mark (Bumguardner 1991). Quality of the OTC marks differed based on the side of the scale viewed under the microscope (i.e., fibrillary plate versus grooved) and were clearer when viewed with the grooved side up. High intensity of the OTC marks enabled scales to be viewed dry, mounted between a glass slide and coverslip.

Back-calculation models and analyses

The Fraser–Lee (i.e., intercept-corrected direct proportion) model is a linear, direct proportional back-calculation method (Fraser 1916; Lee 1920) that back-calculates length for individual fish as

$$L_i = a + (L_c - a)(S_i/S_c)$$

where L_i is BCL at annulus i, L_c is length at capture, S_i is scale (bony structure) radius to annulus i, S_c is total scale (bony structure) radius, and a is a correction factor.

The correction factor a proposed by Fraser (1916) represents the length of the fish at the time of scale formation. The correction factor is commonly derived empirically from observations of larval and juvenile fish (Everhart 1948) or mathematically from the y-intercept of a linear regression of body length on scale radius (Lee 1920; Whitney and Carlander 1956; Carlander 1982) and hereafter is referred to as an "intercept." No intercept was used for otolith back-calculations because otoliths are present at hatching or soon thereafter (Taubert and Coble 1977; Schramm et al. 1992). A zero-intercept reduces the Fraser–Lee model to the direct proportional or Dahl–Lea model (Dahl 1907; Lea 1910) for mean BCLs. For Fraser–Lee back-calculations from scales, we used the 20-mm bluegill standard intercept value recommended by Carlander (1982). A 20-mm intercept provided accurate BCLs and was recommended as a standard for hybrid sunfish (Klumb et al. 1999*a*).

Campana (1990) proposed a modification to the Fraser–Lee model, known as the biological-intercept model, to account for systematic variation in the fish length – otolith size ratio with somatic

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Fig. 1. (A) Anterior, dorsal, and posterior axes measured on whole otoliths and (B) anteriolateral axis measured on scales for back-calculation.

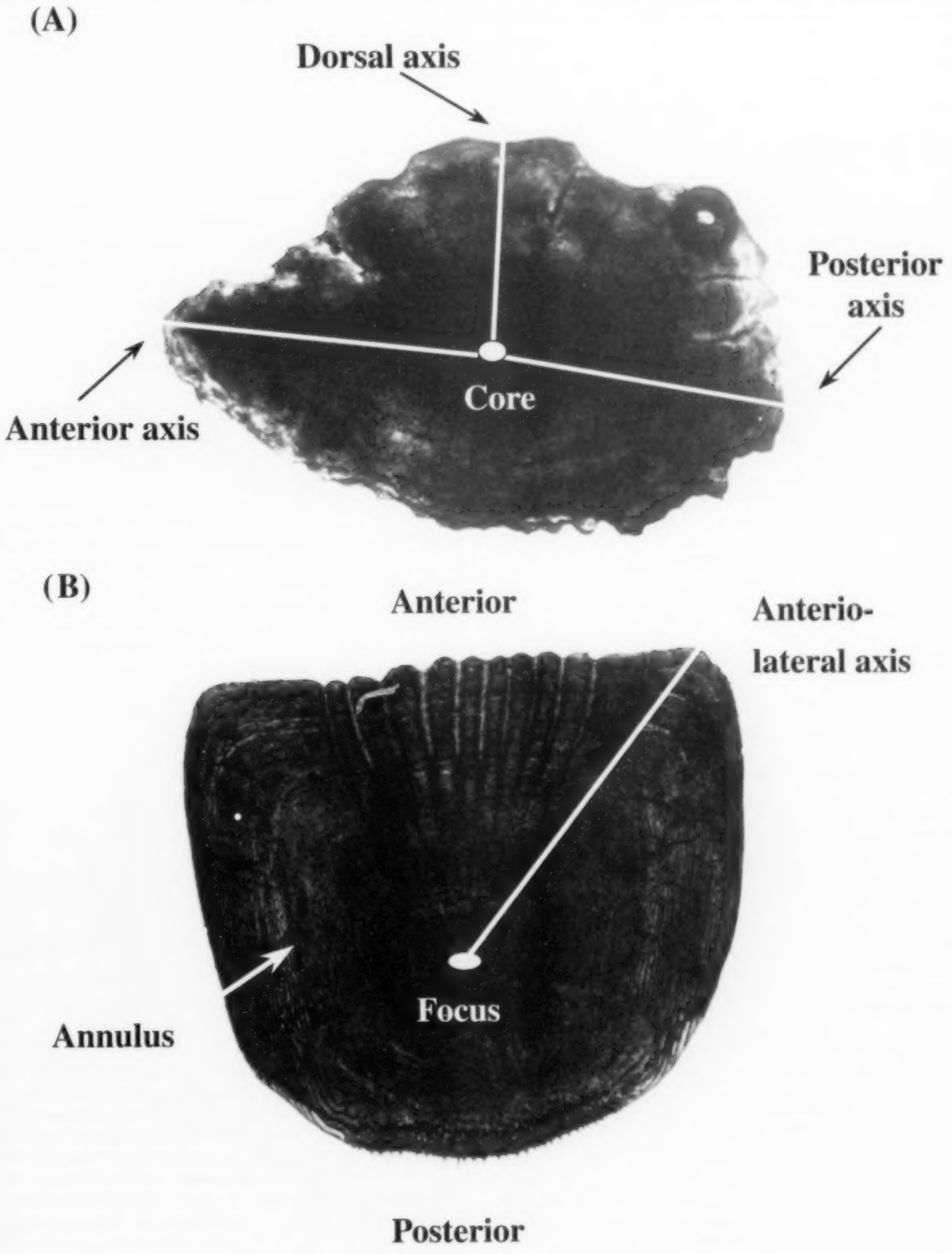

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Table 2. Numbers of OTC-marked hybrid sunfish with scales and otoliths used for back-calculation.

OTC mark	Scales	Otoliths		
		Anterior axis	Dorsal axis	Posterior axis
1	83	40 L, 47 R	67 L, 61 R	63 L, 52 R
2	83	40 L, 47 R	67 L, 61 R	63 L, 52 R
3	28	13 L, 14 R	22 L, 16 R	17 L, 15 R

Note: Left (L) and right (R) otoliths were analyzed separately along three axes.

growth rate. Essentially, the Fraser–Lee model is corrected along both the x- and y-axes using biologically determined intercepts corresponding to the onset of proportional growth between the body and bony structure. Campana's (1990) biological-intercept model back-calculates length for individual fish as

$$L_i = L_c + (L_c - L_0)(S_i - S_c)/(S_c - S_0)$$

where L_i is BCL at annulus i, L_c is length at capture, S_i is scale (bony structure) radius to annulus i, S_c is total scale (bony structure) radius, L_0 is a correction factor for body length, and S_0 is a correction factor for bony structure length. For otoliths, we used the corrections of Hales and Belk (1992) corresponding to a total length of 6 mm (L_0) for bluegill larvae at swim-up and an otolith radius at swim-up of 0.04 mm (S_0) found by Taubert and Coble (1977) for the posterior axis. We used this correction factor for all three axes. For scale back-calculations, proportionality is generally assumed to start at scale formation. Bluegills attain a body length of approximately 20 mm at scale formation (Potter 1925), and we set $S_0 = 0$; this results in Campana's model being identical to the original Fraser–Lee model.

The Weisberg linear model partitions fish growth into two main effects: growth due to age of the fish and growth due to environmental effects in a given year (Weisberg and Frie 1987; Weisberg 1993) in a general additive ANOVA model:

$$\mu_{aj} = (\text{age effect})_{a+1-j} + (\text{year effect})_j$$

where μ_{aj} is the population mean bony structure increment for fish of age a that grew in year j, a is age, and j is year of growth ($j = 1$ is the most current year of growth).

Back-calculation in the Weisberg model is a multistep process. First, an ANOVA model is fit to bony structure increments to estimate the effects (i.e., coefficients) of age and the environment on mean bony structure increments. A regression of total length on bony structure radius is then calculated and the parameters are derived by maximum likelihood estimation. Length increments are then modeled for age effects and year effects with the coefficients derived by maximum likelihood estimation. Lastly, the estimated mean length increments for each age in a given year are summed to give the total BCL.

Assumptions for the Weisberg model are that (*i*) bony structure and length increments have a joint normal distribution, (*ii*) growth increments for a fish are independent among years, (*iii*) fish in the same year grow independently of each other, and (*iv*) the errors are normally distributed. For the body – bony structure regression, the model assumes that the correlation (ρ) between body and bony structure growth is constant for all years and all age groups in the sample. Therefore, the regression model is fit with a common slope for each age group. An F test checks the assumption that ρ is constant. Each age group can have a unique intercept value. Further details of model calculations are given in Weisberg (1986, 1993) and Weisberg and Frie (1987). Back-calculations were obtained with the XLISP-STAT computer program in a Windows™ environment, version 2.1R3 Alpha 6, Windows™ version 3.0, provided by the Minnesota Sea Grant Program (S. Weisberg, Department of Applied Statistics, University of Minnesota, St. Paul, MN 55108-6042, U.S.A., unpublished manual).

The Weisberg model requires at least two year-classes with a minimum of three fish in each year-class in order to run. Because between the second and third OTC treatments, a defective heater electrocuted approximately 63% of the fish, the electrocuted fish and surviving fish were treated as two artificial year-classes. Since both groups of fish were actually reared together (up to the time of the accident), no significant year effects were expected.

For all three models, lengths measured at the time of OTC marking were compared with their respective BCLs. Model error was quantified as the percent difference of BCLs from known lengths. To prevent cancellation of positive and negative errors, absolute values were used to calculate the overall mean percent difference. The slope of the line for an individual fish in the Fraser–Lee model has its natural variability constricted due to forcing the line through a common y-intercept and results in the variance structure being unknown (Weisberg and Frie 1987; DeVries and Frie 1996). Therefore, Fraser–Lee BCLs were tested against known lengths with the Mann–Whitney U test. Statistical analyses could not be performed for the Weisberg model because it does not back-calculate for individual fish. To assess whether otolith and scale increments remained constant after formation (i.e., Francis' (1990) first validation requirement), we compared otolith and scale radii for the electrocuted and surviving fish at the first two OTC marks. All statistical analyses were performed with SPSS for Windows™ release 6.1.2. Statistical significance was set at $\alpha = 0.05$.

Results

OTC marking success

Multiple OTC marking success of hybrid sunfish otoliths was lower than for scales. However, marking success in otoliths and scales was 100% for the first two marks for the 38 hybrid sunfish that survived all three treatments. Three OTC marks were visible in either the left or right otolith and along at least one axis for 61% of the 38 fish that survived the experiment (Fig. 2). For scales, three OTC marks were observed in 74% of the fish surviving all OTC treatments (Fig. 3). Hybrid sunfish growth rates declined with each successive treatment (Table 1); growth declined 47% from the first to second increments and 66% from the second to third increments. Sample size for otolith back-calculations differed for the otolith analyzed (right or left) and axis measured due to nonvisible OTC marks along a particular axis and chipped or cracked otoliths (Table 2). A total of 86% (83) of fish possessed the expected number of OTC marks and had nonregenerated scales usable for back-calculation (Table 2).

Back-calculation model validation

Otoliths

Otolith radii measured to the first two OTC marks were not significantly different between fish electrocuted after the second marking period and fish surviving all three OTC treatments for left and right otoliths along all three axes (Table 2). These results indicate that we met Francis' (1990) first requirement for back-calculation model validation; once formed, otolith radii from the core to the first two OTC marks remained constant. Furthermore, the potential error in back-calculating lengths using whole otoliths due to refractive distortion was minimal; differences between otolith radii

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for electrocuted and surviving fish at both OTC marks were <4%.

The accuracy of Fraser–Lee BCLs based on whole otoliths differed for both the measurement axis used and the OTC mark being analyzed; errors ranged from –8.2 to 7.8%. Overall, no axis consistently provided the most accurate BCLs (Fig. 4). Using the anterior axis, BCLs were significantly different from the actual lengths at the third OTC mark on both right and left otoliths and at the second mark on only the right otolith. BCLs for both the dorsal and posterior axes were significantly different from the actual lengths at the first and third OTC marks on right and left otoliths. There were no significant differences between BCLs using left or right otoliths along all three axes (Mann–Whitney U test: $P > 0.05$). The largest difference between BCLs from left and right otoliths was 4.1 mm for anterior radii measured to the third OTC mark.

The accuracy of BCLs from the biological-intercept model using whole otoliths also differed for both the measurement axis and the OTC mark being analyzed. Errors ranged from –8.0 to 8.2%, and again, no axis consistently provided the most accurate BCLs (Fig. 4). Significant differences between BCLs and the actual lengths at marking occurred for the same axes and OTC marks as the Fraser–Lee model (Fig. 4): the second and third OTC marks using the anterior axis and the first and third marks using the dorsal and posterior axes. There were no significant differences between BCLs using left or right otoliths along all three axes (Mann–Whitney U test: $P > 0.05$). Comparing BCLs from the Fraser–Lee and biological-intercept models resulted in no significant differences (Mann–Whitney U test: $P > 0.05$).

The accuracy of Weisberg BCLs from otoliths differed between measurement axes and OTC marks; errors ranged from –6.5 to 14.3%. No otolith axis consistently provided the most accurate BCLs (Fig. 4). Differences between BCLs derived from left or right otoliths were small; the largest difference was 6 mm for posterior radii measured to the third OTC mark.

Weisberg model assumptions were not met for all otolith axes, and the otolith increment ANOVA model detected one nonexistent year effect. The constant correlation assumption between otolith and body increments was met for the anterior axis and dorsal axis for right and left otoliths. Along the posterior axis of right and left otoliths, the constant correlation assumption was violated (F test: right $F = 5.3427$, df = 1,48, $P = 0.02$; left $F = 7.8419$, df = 1,59, $P = 0.01$). Age effects for the otolith increment ANOVAs were significant for all axes and otoliths. The only unexpected significant year effect from the ANOVAs was for left otoliths measured along the anterior axis.

The relative magnitude of over- and underestimation at all three OTC marks was remarkably similar for the Fraser–Lee, biological-intercept, and Weisberg models for all three otolith axes (Fig. 4). For all three models, back-calculations overestimated the known length at the first mark and underestimated length at the second and third marks. Lowest differences for otolith BCLs from actual lengths were always seen at the second OTC mark. Weisberg BCLs from dorsal and posterior measurements overestimated known length by >10% for the first OTC mark. No BCLs from otoliths differed from the known length by >8.5% for the Fraser–Lee or biological-intercept model.

Scales

Scale radii measured to the first two OTC marks were not significantly different between fish electrocuted after the second marking period and fish surviving all three OTC treatments (Table 3) and indicate that we met Francis' (1990) first requirement for back-calculation model validation. Differences between scale radii for electrocuted and surviving fish at both OTC marks were <2%.

For the Fraser–Lee model, the BCLs from scales underestimated the actual length at marking by 0.6–4.3% (Fig. 4). The overall mean percent difference of BCLs from the actual length at marking was 1.8% (2.3 mm). The BCLs were significantly smaller (4.3%, 5.1 mm) than the actual lengths at marking only at the first OTC mark.

Error for Weisberg model BCLs from scales ranged from –2.2 to 0.6% (Fig. 4). Across all marks, the overall weighted mean difference of Weisberg BCLs was 1.4% (1.8 mm) less than the actual lengths at marking. The constant correlation assumption between scale and body increments, requiring equal slopes for the age groups, was met (F test: $F = 0.0459$, df = 1,79, $P = 0.83$). For the Weisberg scale increment ANOVA, age effects were significant but year effects, as expected, were not.

Neither the Fraser–Lee nor the Weisberg model consistently provided more accurate estimates of the actual lengths from scale radii of the OTC-injected hybrid sunfish. However, the Fraser–Lee model did consistently underestimate length. The overall BCL mean percent differences from the actual lengths at OTC marking for both models were similar, 1.8% for the Fraser–Lee model and 1.4% for the Weisberg model.

OTC mark width and scale measurement error

Despite using identical concentrations (by percent body weight) of OTC for each injection, the width of marks was not uniform for successive marking attempts in the anteriolateral region of the scale (Table 4). The first mark was significantly wider than the second and third marks (one-way ANOVA: $F = 7.888$, $P = 0.001$). The widest OTC marks were formed before the interval with the highest growth rate (Table 1). The OTC mark widths declined 29.4% from treatment 1 to treatment 2.

The mean width of the OTC fluorescence was 0.054 mm; error as a percentage of the total scale radius for marks 1–3 was 1.4% (Table 4). The difference between Fraser–Lee BCLs using radii measured to the inner versus outer OTC mark edges was 1.7, 1.5, and 1.2 mm for marks 1–3, respectively. The overall mean difference between BCLs was 1.5 mm. The ratio of the mean change in Fraser–Lee BCLs to the percentage of the scale radius within the OTC mark (i.e., known error) was 1.041 mm to 1% error. A scale measurement error of 1% on Weisberg BCLs resulted in similar differences between the BCLs of 1.7, 1.5, and 1.1 mm for marks 1–3, respectively. The mean difference between Weisberg BCLs was also 1.5 mm. For the Weisberg model, the ratio of the mean change in BCLs to the percentage of the scale radius in the OTC mark was the same as for the Fraser–Lee model, 1.041 mm to 1% error.

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Fig. 2. Whole right otolith from a 191-mm hybrid sunfish showing three OTC marks in the three regions measured for back-calculation: (A) anterior region, (B) dorsal region, and (C) posterior region.

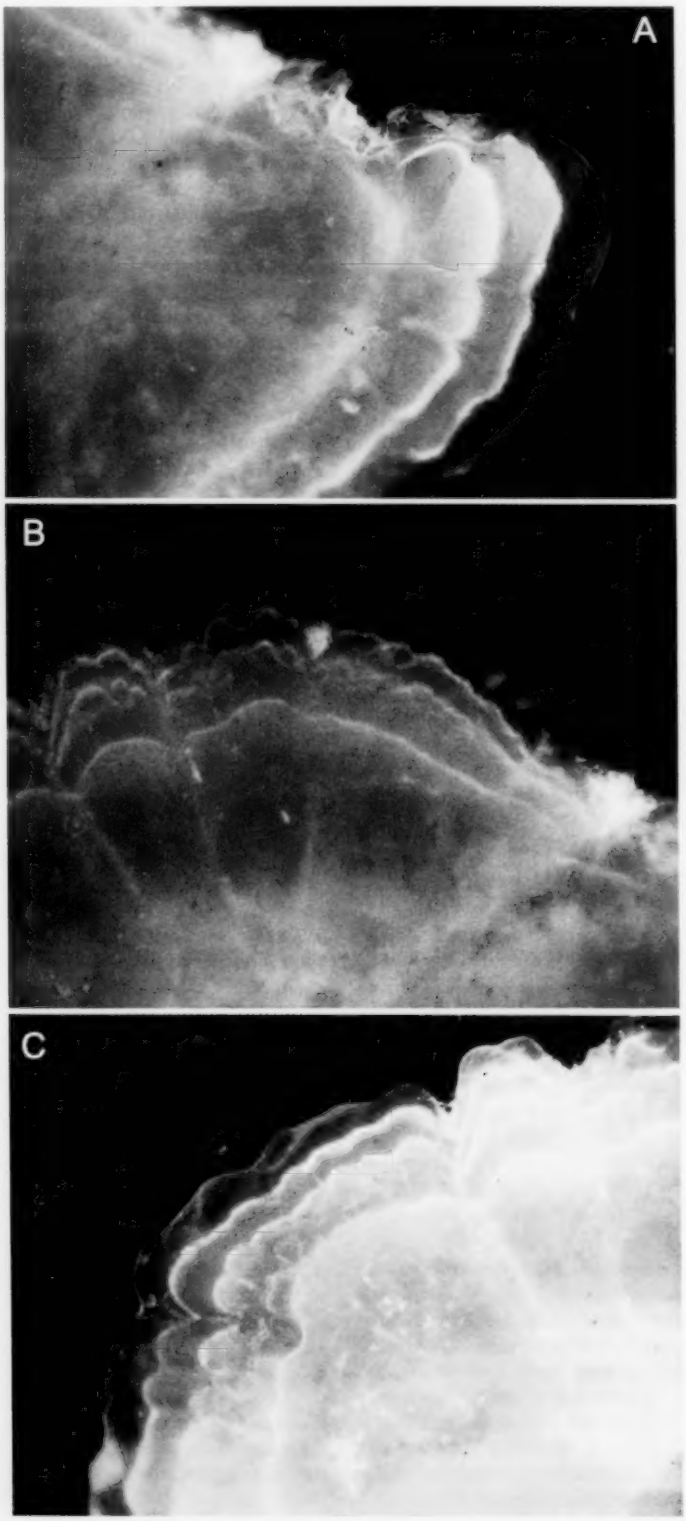

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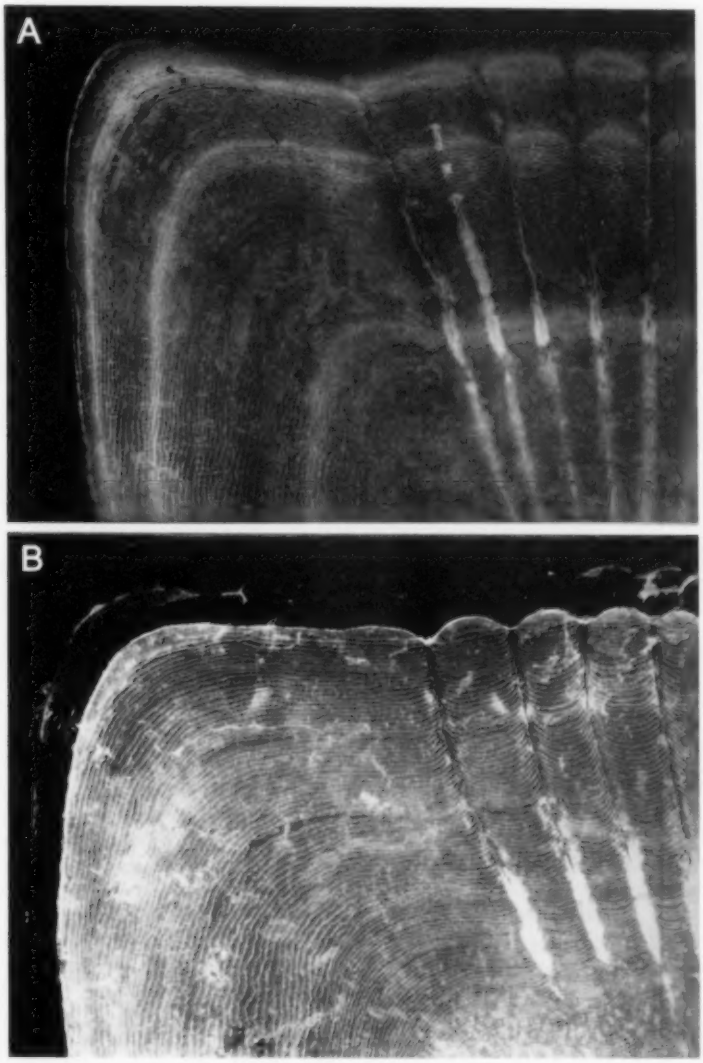
Fig. 3. Comparison of experimental and control bluegill scales indicating the presence and absence of OTC marks. (A) Scale from a 198-mm hybrid sunfish injected with OTC three times and exhibiting three fluorescent marks; (B) scale from a 175-mm control fish not injected with OTC.



Discussion

OTC marking success

Injection is a more direct method of administering OTC than feeding or immersion (Strasdine and McBride 1979), and marking success in otoliths and scales was 100% for the first two marks for the 38 hybrid sunfish that survived all three treatments. Even though actual growth rates decreased from treatment 1 to treatment 3, variability of mark intensity was low. Seepage of OTC out of the injection wound and misidentification of four control fish affected marking success. Marking success of OTC-injected fish is generally high and consistent for scales and otoliths from the same fish (Campana and Neilson 1982; Babaluk and Craig 1990). The low marking success that we observed for otoliths may be due to using whole otoliths; use of sectioned otoliths may have increased our overall marking success.

Back-calculated growth

In this study, OTC marks created in laboratory fish at 90-day intervals simulated annuli and removed the possibility of back-calculation error due to incorrect age assessment. These OTC marks met the second requirement for validation of a back-calculation technique (Francis 1990) by removing concern for the validity of natural annuli formation. We found that once formed, the distance between OTC marks remained constant for hybrid sunfish otoliths and scales, Francis' (1990) first validation requirement. Klumb et al. (1999*b*) found that scale radii between annuli remained constant for individually marked smallmouth bass (*Micropterus*

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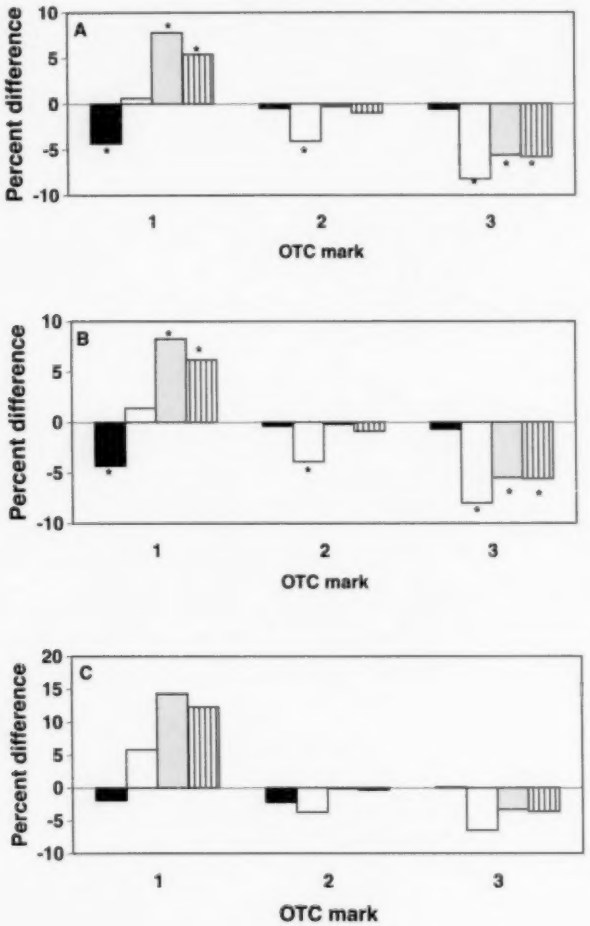
Fig. 4. Percent differences of BCLs from the observed lengths of hybrid sunfish at the time of OTC marking for the (A) Fraser–Lee, (B) biological-intercept, and (C) Weisberg models. Black bars, scales; open bars, otoliths along the anterior axis; shaded bars, otoliths along the dorsal axis; hatched bars, otoliths along the posterior axis. Asterisks indicate significant differences between observed lengths and BCLs (Mann–Whitney U test: $P < 0.05$). Only data for right otoliths are provided. Scale BCLs for the Fraser–Lee and biological-intercept models are identical.



dolomieu) and walleye (*Stizostedion vitreum*) captured multiple times. As a result, this study focused on how well the back-calculation models estimated past growth.

Otoliths from OTC-injected hybrid sunfish provided less accurate back-calculations than scales for the Fraser–Lee, biological-intercept, and Weisberg models. For all models, none of the three otolith axes consistently provided better length estimates, yet the three models may have detected a similar change in otolith growth along each specific axis with respect to body growth. The otolith to body relationship has been found to vary with season (Rijnsdorp et al. 1990), and Casselman (1990) found that otoliths grew slower than the body during rapid somatic growth. Also, otolith growth has been observed to "decouple" or lag behind changes in somatic growth in juvenile fishes in the laboratory (Wright et al. 1990; Bradford and Geen 1992). The possible decoupling of otolith and somatic growth may explain the greater error in back-calculations for otoliths than for scales in this study. Otolith growth also follows water temperature more closely than ration level (Bradford and Geen 1992). Bradford and Geen (1992) observed decoupling in experiments on juvenile chinook salmon (*Oncorhynchus tshawytscha*) lasting 70 days with temperatures varied by 5–8.6°C. In this study, water temperature varied approximately 5°C between the 90-day growth intervals, and these changes may have produced decoupling of otolith and somatic growth in our juvenile hybrid sunfish.

The biological-intercept model resulted in otolith BCLs that were not significantly different from BCLs derived from the Fraser–Lee model without an intercept (the Dahl–Lea model) because the small biological-intercept corrections used for larval body and otolith size at swim-up had little effect on estimated growth for the 90-day intervals in this study. However, the biological-intercept modifications have been found to increase accuracy for larval daily growth estimates. Sirois et al. (1998) found that daily growth estimates

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Table 3. Results of *t* tests (assuming equal variance) comparing hybrid sunfish mean ± 2 SE otolith and scale radii (mm) measured to the first and second OTC marks for fish killed (electrocuted) after the second marking attempt and fish surviving all three OTC marking treatments.

OTC mark	Electrocuted fish	Surviving fish	*t*	*P*
Right otolith, anterior axis				
1	2.40±0.07	2.36±0.12	2.0141	0.56
2	3.32±0.04	3.36±0.15	2.0141	0.95
Left otolith, anterior axis				
1	2.35±0.06	2.26±0.16	2.024	0.24
2	2.85±0.09	2.87±0.16	2.024	0.80
Right otolith, dorsal axis				
1	1.54±0.04	1.55±0.10	2.0010	0.79
2	1.81±0.05	1.84±0.09	2.0010	0.60
Left otolith, dorsal axis				
1	1.54±0.04	1.49±0.07	1.9971	0.24
2	1.80±0.05	1.79±0.07	1.9971	0.68
Right otolith, posterior axis				
1	2.28±0.08	2.37±0.13	2.0086	0.24
2	2.74±0.10	2.82±0.12	2.0086	0.40
Left otolith, posterior axis				
1	2.29±0.06	2.23±0.16	1.9996	0.36
2	2.74±0.07	2.69±0.16	1.9996	0.54
Scales				
1	2.40±0.07	2.36±0.12	1.9897	0.56
2	3.32±0.04	3.36±0.15	1.9897	0.64

Note: Scale and otolith axes measured are indicated in Fig. 1 and sample sizes are provided in Table 2.

of known-age larval rainbow smelt (*Osmerus mordax*) were within 16.3% of the observed growth trajectories, but periods of zero or negative growth for larval fish are overestimated by the biological-intercept model (Secor and Dean 1992). The biological-intercept model can also reduce or remove the effect of Lee's phenomenon in annual BCLs (Campana 1990).

In other studies, validation with individually tagged fishes found Dahl–Lea or Fraser–Lee otolith back-calculation errors generally within 15% of observed lengths. For Atlantic cod (*Gadus morhua*) reared in pens with induced fast, slow, or variable growth, Smedstad and Holm (1996) found that all Fraser–Lee BCLs were within 10% of known lengths during a 2.5-year study. Holmgren (1996 found Dahl–Lea back-calculation errors of 15% or less for OTC-marked and individually tagged European eels (*Anguilla anguilla*) despite violation of the assumption of direct proportional growth between the otolith and body. For largemouth bass (*Micropterus salmoides*), Howells et al. (1995) found Dahl–Lea BCLs errors of –6.8 to 9%. Hales and Belk (1992) found differences of 1.5–10.6% between BCLs with the biological-intercept model and the actual lengths of bluegill aged 1–9; the greatest error (>9%) was seen at the youngest ages (ages 1–3).

Verification of BCLs from the Dahl–Lea or Fraser–Lee model by other studies for other centrarchids indicates that these models can provide reasonable length estimates from otolith annuli. However, because individually marked fish or groups of fishes with known growth were not used, these studies do not constitute complete validation. Schramm et al. (1992) compared otolith BCLs using the Dahl–Lea and Fraser–Lee methods for largemouth bass and found more accurate BCLs for the Dahl–Lea model compared with the Fraser–Lee model. Heidinger and Clodfelter (1987) found no significant differences between empirical lengths, Dahl–Lea BCLs, and Fraser–Lee BCLs of age-2 to age-4 smallmouth bass. For white crappie (*Pomoxis annularis*), age-1 to age-3 length frequencies from spring samples corresponded well to Fraser–Lee BCLs (Maceina and Betsil 1987). Schramm and Doerzbacher (1985) found a similar concordance of age-2 and age-3 Fraser–Lee BCLs and length frequencies of black crappie (*Pomoxis nigromaculatus*); however, 52% of the age-1 BCLs were below the minimum size captured in spring trawl samples. The discrepancy observed for age-1 black crappie by Schramm and Doerzbacher (1985) may be due to the size selectivity of their trawl.

For hybrid sunfish, segregation of right and left otoliths for back-calculation was unnecessary. No significant differences were observed for Fraser–Lee or biological-intercept model back-calculations from right and left otoliths. Boxrucker (1986) found similar results for Fraser–Lee otolith back-calculations for white crappie. Although not statistically tested, differences of Weisberg BCLs for right and left otoliths were all within 6.5 mm of each other for the OTC-injected hybrid sunfish.

For back-calculations from scales, both the Fraser–Lee and Weisberg methods produced estimates very close to the actual lengths. Although statistically significant, the differences of Fraser–Lee model BCLs from the actual lengths at the first OTC mark were <4.3%. Weisberg BCLs were closer to the actual lengths; all differences were <2.2%. However, the simpler Fraser–Lee model was found to be more precise than the Weisberg model, especially for younger ages, and was recommended for back-calculating growth of wild smallmouth bass and walleye (Klumb et al. 1999*b*). In this study, the observed error of both models likely lies within the range of body length measurement error (Gutreuter and Krzoska 1994).

The greatest error for BCLs from scales was seen at the first OTC mark; both linear models failed to accurately describe the early growth of these fishes. A similar result for the Fraser–Lee model was found by Klumb et al. (1999*a*) in another experiment with OTC-marked bluegill and hybrid sunfish. Since our fish were anesthetized during measurement and the mean radii of three scales was used for back-calculation, violation of the isometric assumption of scale and body growth is the most likely source of the greatest errors seen at the earliest OTC mark. The body–scale relationship for early fish growth is generally nonlinear (Regier 1962). An allometric body–scale relationship is only approximated with the linear Fraser–Lee, biological-intercept, and Weisberg models, necessitating caution when interpreting the earliest BCLs.

Validation studies (Davies and Sloane 1986; Howells et al. 1995; Klumb et al. 1999*b*) of older marked fishes in the wild indicate that Fraser–Lee BCLs from scales generally provided realistic estimates of historic fish growth. BCLs of individually tagged smallmouth bass and walleye underestimated the actual length at marking by 10% or less (Klumb et al. 1999*b*). Davies and Sloane (1986) found that Fraser–Lee

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Table 4. Width of OTC marks, percentage of the total scale radius within the mark, and mean number of circuli in the fluorescent band in the anteriolateral region of scales from 30 randomly selected hybrid sunfish injected with OTC.

OTC mark	*N**	Mean width ± 2 SE (mm)	Mean width as percentage of total radius ± 2 SE	Width range (mm)	Mean number of circuli ± 2 SE
1	30	0.068±0.0098 *a*	1.86±0.27	0.030–0.147	2.9±0.42
2	30	0.048±0.0056 *b*	1.28±0.13	0.020–0.098	2.3±0.24
3	9	0.048±0.0064 *b*	1.18±0.28	0.029–0.078	2.7±0.40

Note: Widths followed by different letters are significantly different (one-way ANOVA, Bonferroni multiple-range test: $P < 0.0008$).

*Sample size differs because not all fish survived to the third OTC treatment.

BCLs underestimated length at release for hatchery-reared rainbow trout (*Oncorhychus mykiss*) by 5% or less. For individually tagged largemouth bass (ages 3–8), Howells et al. (1995) found that Dahl–Lea BCLs underestimated known lengths in October by 2.4–21.4%, with the greatest errors (>8%) seen at ages 3 and 4. These large errors seen by Howells et al. (1995) at the young ages are likely due to not using an intercept in the Fraser–Lee model in conjunction with possible late season growth before annulus formation. Additional verification for Fraser–Lee BCLs from scales of bluegill (Regier 1962) and pumpkinseed (*Lepomis gibbosus*) (Pierce et al. 1996) found that all length estimates were within 5% of measured lengths for other fish captured at the start or end of the growing season.

In our study with juvenile hybrid sunfish, we found that scale BCLs were more accurate than those from otoliths. Only one other validation study (Howells et al. 1995) compared Dahl–Lea BCLs from scales and otoliths. The range of errors that they found differed from our study in that error of otolith BCLs (–6.8–9%) was narrower than that of scale BCLs (–21.4 to 2.4%), indicating greater precision of BCLs from otoliths versus scales of largemouth bass (ages 3–8). The errors for scales likely would have been smaller if Howells et al. (1995) had used the Fraser–Lee model with an intercept for scale BCLs. In a study that verified BCLs to empirical lengths of three species (ages 1–4) caught in April at the start of the growing season, otolith BCLs using the Dahl–Lea model and Fraser–Lee BCLs for scales were not significantly different from the observed lengths (Heidinger and Clodfelter 1987). However, for long-lived species, age assessments from scales generally can become unreliable, resulting in greater growth estimation errors (Howells et al. 1995).

OTC mark width and scale measurement error

The approximately 2.5 circuli occurring within the fluorescent band of the OTC mark in the scales from our hybrid sunfish concurred with other reported OTC mark widths. Kobayashi et al. (1964) reported that approximately two or three "ridges" were in the tetracycline mark in the anterior region of goldfish (*Carassius auratus*) scales. The red drum (*Sciaenops ocellatus*) scale pictured in Bumguardner (1991) showed two circuli in the bright OTC band.

Due to the width and "bleeding" of OTC marks, the specific location used to measure scale radii for back-calculations could potentially affect the accuracy of those length estimates. However, differences between BCLs for the Fraser–Lee and Weisberg models using scale radii measured to the inside and outside edges of the OTC marks were negligible (<3 mm). Moreover, this small change in BCL also indicates that uncertainty of where a natural annulus starts with respect to the scale focus would not greatly affect length estimates for both models if scale radii are measured within 1% of the true annulus. However, the fish used in our study were less than age 2; misjudging an annulus by 1% on older fishes with crowded annuli near the scale's edge may result in greater back-calculation errors. Smedstad and Holm (1996) increased otolith radii by 3% to study uncertainty in location of the true otolith radius that corresponded to the time of length measurements; they found insignificant changes in Fraser–Lee BCLs. Care should always be taken to accurately measure bony structure radii, but minor errors do not appear to appreciably change length estimates.

In summary, this experiment, along with our other validation studies in the laboratory (Klumb et al. 1999*a*) and field (Klumb et al. 1999*b*), provides fisheries researchers and managers information about the range of potential errors that can be expected from back-calculating fish growth. All BCLs in this study for otoliths and scales using the Fraser–Lee, biological-intercept, and Weisberg models were within 15% of the actual lengths. Whether the potential of 15% error in BCLs is tolerable depends on individual research or management objectives. Due to the greater accuracy of scales, we recommend using scales over otoliths for back-calculating growth of juvenile hybrid sunfish. The Fraser–Lee and Weisberg models had similar, small ranges of back-calculation error for scales. However, model choice depends on a study's specific objectives, since growth rates for individual fish cannot be derived with the Weisberg model.

Although otoliths can provide more reliable age assessments for many older fishes (Heidinger and Clodfelter 1987; Howells et al. 1995), back-calculation error was much more variable than for scales in our study with juvenile hybrid sunfish. Further detailed study of otolith – body growth relationships, beyond mere otolith–length on body–length regressions, is needed to improve growth estimates from these structures. At present, we recommend the simpler Fraser–Lee model (without an intercept) or biological-intercept model for otolith back-calculations over the more complex Weisberg model. However, the potential decoupling of the otolith–body relationship and its effect on BCLs needs further study, which necessitates caution when interpreting otolith back-calculations for juvenile fish. Species-specific validation of back-calculation model performance should be attempted where possible, as has been recommended for techniques used to assess fish ages (Beamish and McFarlane

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1983). With further validation of back-calculation models for additional fish species, life stages, and aging structures, greater confidence can be placed in data derived by this popular and powerful fisheries tool.

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Habitat-, season-, and size-specific variation in diel activity patterns of juvenile chinook salmon (*Oncorhynchus tshawytscha*) and steelhead trout (*Oncorhynchus mykiss*)

Michael J. Bradford and Paul S. Higgins

Abstract: Juvenile salmonids that live in streams are sometimes nocturnal and may spend the day concealed in the stream substrate. We observed the diel activity patterns of juvenile chinook salmon (*Oncorhynchus tshawytscha*) and steelhead trout (*Oncorhynchus mykiss*) in two areas of the Bridge River, British Columbia, in all four seasons. In a reach with higher flows, most fish were nocturnal year-round, and they emerged from the substrate only at dusk to forage. In the reach with lower flows, some fish were active in the water column in the day in summer, but others remained concealed in the substrate until dusk. Parr and older fish were more nocturnal in summer than fry. All fish were nocturnal in winter. Because our study design controlled for temperature and photoperiod, we concluded that the differences in behaviour that we observed between reaches were due to habitat conditions that likely affected the trade-off between more risky daytime foraging and less efficient, but safer, nighttime foraging. Habitat-driven variation in activity patterns will likely affect the processes that regulate these populations and could make the prediction of the effects of ecosystem manipulations such as changes in flow very difficult.

Résumé : Les saumons juvéniles qui vivent dans les cours d'eau sont quelquefois nocturnes et peuvent passer la journée cachés dans le substrat. Nous avons observé en toute saison les profils journaliers d'activité de juvéniles du Saumon quinnat (*Oncorhynchus tshawytscha*) et de la Truite arc-en-ciel anadrome (*Oncorhynchus mykiss*) dans deux sections de la rivière Bridge en Colombie-Britannique. Dans la section à débits plus élevés, la plupart des poissons sont nocturnes toute l'année et ils ne quittent le substrat qu'au crépuscule pour chercher leur nourriture. Dans la section à débits plus faibles, quelques poissons s'activent dans la colonne d'eau pendant la journée en été, mais d'autres restent cachés dans le substrat jusqu'au crépuscule. Les tacons et les poissons plus âgés sont plus nocturnes en été que ne le sont les alevins. Tous les poissons sont nocturnes en hiver. Parce que notre plan expérimental permet de tenir compte de la température et de la photopériode, nous concluons que les différences de comportement que nous avons observées dans les deux sections sont dues à des conditions d'habitat qui affectent le compromis entre la quête de nourriture de jour plus périlleuse et la quête de nuit plus sûre, mais moins efficace. Les variations dans les profils d'activité sous contrôle de l'habitat affectent vraisemblablement les processus qui assurent la régulation de ces populations; il peut alors devenir très difficile de prédire les effets de manipulations de l'écosystème, telles que des changements de débit.

[Traduit par la Rédaction]

Introduction

Juvenile salmon living in freshwater face at least two challenges: to grow to as large a size as possible, as survival in both freshwater and ocean habitats is often size dependent (Hunt 1969; Holtby et al. 1990), and to avoid being eaten. Unfortunately, when fish forage, they are usually exposing themselves to some risk of predation so that there is a trade-off between energy acquisition and safety (Lima and Dill 1990). For stream-dwelling salmonids, predation risk is often managed through the use of cover, either in the form of physical refugia from predators or in the form of habitats in which predators are inefficient, such as in turbid water or darkness. Fish must then venture from cover or refuge habitat to forage, and decisions that are made about the time spent either in cover or foraging can have major impacts on both individual fitness and population dynamics (Walters and Juanes 1993).

A very conspicuous aspect of the activity patterns of stream fish is the daytime use of the stream substrate as cover. Many species of stream-dwelling salmon and trout (*Salmo* spp., *Oncorhynchus* spp.) switch from diurnal activity during the warmer months to nocturnal activity in winter (Campbell and Neuner 1985). When water temperatures decline below approximately 8–12°C, fish are often observed to begin to conceal themselves in interstitial refuges within the stream channel during the day and emerge only at dusk to feed

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(Riehle and Griffith 1993). This behaviour may not be restricted to winter. Hillman et al. (1992) and Fraser et al. (1995) found that salmon and trout were concealed at low temperatures in summer and fall, and others have noted that juvenile salmonids are difficult to observe, regardless of season, at low temperature (Adams et al. 1988). Gries et al. (1997) found larger (greater than age 1) Atlantic salmon (*Salmo salar*) juveniles were nocturnal, even at temperatures in excess of 20°C.

Recently, diel activity patterns of stream-dwelling fish have been viewed in the context of individuals making decisions about the trade-off between foraging and the risk of predation. A useful construct is the "minimize u/g or u/f" rule (Metcalfe et al. 1999), which suggests that fish should occupy habitats that minimize the ratio of mortality risk (u) to growth (g) or feeding (f). During the day, juvenile salmonids are more efficient foragers than at night (Fraser and Metcalfe 1997), but they may be even more vulnerable to predation from visually based piscivores (Metcalfe et al. 1999). Thus, there may be circumstances where crepsucular or nighttime foraging minimizes u/g compared with daytime activity (Clarke and Levy 1988). In streams, the crepuscular period may be particularly important because invertebrate drift often increases at dusk, providing potentially good foraging conditions at light levels that reduce the risk of predation (Rader 1997).

The minimize u/g rule also predicts that activity patterns might vary among habitats in a stream if there are consistent habitat- and time-of-day-specific differences in the risks of predation or foraging opportunities. Viewed in this context, activity patterns will not necessarily correlate with simple environmental factors such as temperature or length of day (i.e., season) but might reflect a more complex interaction between an individual and its habitat.

Activity patterns might also vary among individuals within a population. If body size at a certain time and subsequent survival are related (e.g., for smolting or overwintering), there is likely an optimal balance between foraging and predation risk to ensure that an individual reaches sufficient size by the end of the growing season. Theory predicts that smaller individuals should forage more and thus expose themselves to more risk and that the risk that small individuals are willing to assume should increase with time as the need to gain mass before the end of the season becomes increasingly critical. The "asset protection principle" of Clarke (1994) proposes that an individual that is especially large at a certain time would try and protect its "assets" (i.e., body mass) by taking a lower risk strategy. In our context, the principle predicts that larger fish should be more nocturnal; this prediction has been confirmed in experimental studies on overwintering juvenile Atlantic salmon (Metcalfe et al. 1998). But the opposite result was obtained by Gries and Juanes (1998), who found that older age-classes of juvenile Atlantic salmon were less nocturnal than young-of-the-year fish.

State-dependent activity patterns might also operate at a much smaller time scale than over the length of the growing season. Short-term variation in foraging success, hunger, or physiological state might result in the decision to be diurnal or nocturnal being made on a daily basis. In this case, activity might be less dependent on size than is predicted for the asset protection principle. The experiments of Bull et al. (1996) and Metcalfe et al. (1999) on overwintering fish support this idea, as they found that individuals that were starved or were losing weight were more likely to be day active than well-fed or growing fish.

In this study, we describe year-round variation in activity patterns of juvenile chinook (*Oncorhynchus tshawytscha*) and steelhead trout (*Oncorhynchus mykiss*) in the Bridge River, a flow-regulated tributary of the Fraser River, British Columbia. Our study is unique in that there is a wide range of habitat conditions in the Bridge River over a very short distance, and thus, we can evaluate the effects of habitat on behaviour, independent of confounding effects of photoperiod, weather, genetic origin, or other inherent differences in the fish. We used our field observations to refute the idea that variation in activity patterns is driven by simple proximal factors such as temperature or photoperiod. We suggest that activity patterns that we saw should be viewed as examples of animal decision making under predation risk (Lima and Dill 1990) which can be expected to vary with environmental conditions that alter the trade-off between energy gains from foraging and the risk of predation. Finally, we evaluated a basic prediction of the asset protection principle, that activity should be size dependent, to help us understand the underlying cause of patterns in activity that we saw.

Materials and methods

Study area and habitat conditions

The Bridge River is a tributary of the Fraser River and is located on the eastern side of the Coast Mountains of British Columbia. The Bridge River is impounded 40 km upstream of its confluence with the Fraser River by Terzaghi Dam, and all inflows above the dam are diverted to an adjacent watershed. The river channel is confined by mountain slopes and has a relatively steep gradient (0.7–3%); the channel substrate is composed predominantly of large boulders and cobble (see Higgins and Bradford (1996) for more details and a map).

The upper part of the Bridge River, 4–15 km below the dam (the first 4 km below the dam are dry), is characterized by low and relatively stable flows. Groundwater and five small tributaries supply the upper area with a mean annual discharge of approximately 0.7 $m^3 \cdot s^{-1}$ (Fig. 1). Fifteen kilometres below the dam, the unregulated Yalakom River joins the Bridge River and supplies on average 4.3 $m^3 \cdot s^{-1}$ (minimum 0.63 $m^3 \cdot s^{-1}$, maximum 35.6 $m^3 \cdot s^{-1}$; Water Survey of Canada gauge 08ME025, 1983–1996) of the flow to the river.

The fish community of the Bridge River is largely juvenile anadromous salmonids that are progeny of adults that spawn throughout the Bridge River. Juvenile chinook salmon and steelhead trout are the most common, and smaller numbers of coho salmon (*Oncorhynchus kisutch*) are also present (Higgins and Bradford 1996). Resident adult rainbow trout are observed throughout the river, but bull trout (*Salvelinus confluentus*) and mountain whitefish (*Prosopium williamsoni*) are mainly restricted to the area below the confluence with the Yalakom River. We observed the following avian and mammalian predators capable of taking juvenile salmon: common merganser (*Mergus merganser*), belted kingfisher (*Ceryle alcyon*), American dipper (*Cinclus mexicanus*), great blue heron (*Ardea herodias*), and mink (*Mustela vison*).

Methods

We chose two representative study reaches that were located 5.0 km upstream and 2.5 km downstream of the confluence of the

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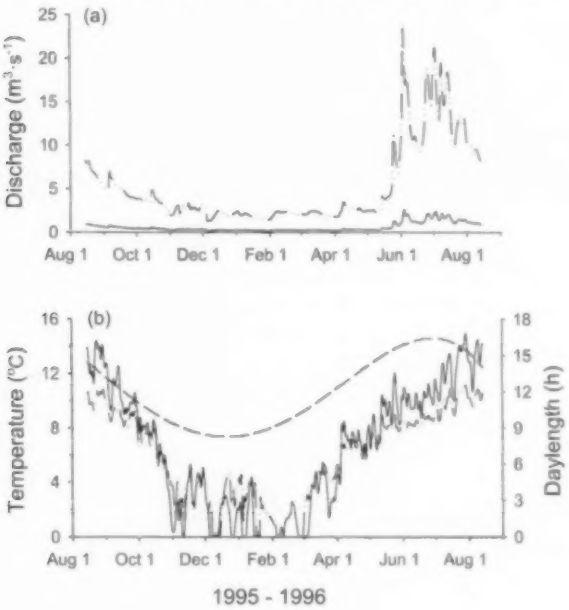
Fig. 1. (*a*) River discharge for the upper (solid line) and lower (dashed-dotted line) reaches of the Bridge River and (*b*) water temperature in the upper (solid line) and lower (dashed-dotted line) reaches and the photoperiod for the region (dashed line).



Yalakom River with the Bridge River. These reaches were chosen because they were relatively close yet had very different physical conditions. Flow in the lower reach was higher, resulting in increased water velocities and a twofold increase in stream depth (Fig. 2).

We recorded stream temperatures by anchoring automated thermographs in about 50 cm of water in each study reach and we collected measurements at 15-min intervals during the study. We also installed light intensity meters (Hobo-LI, Onset Computers Inc.) on the bank at each study reach at sites that were unobstructed by overhanging vegetation. We collected light measurements at 5-min intervals. Light intensity ranged from approximately 0.06 to 12 600 $lm \cdot m^{-2}$, with dusk at approximately 1 $lm \cdot m^{-2}$.

To observe the diel activity patterns of juvenile salmonids, we conducted snorkel surveys in the upper and lower study areas between August 1995 and August 1996. In the upper reach, we observed that juvenile salmon and trout were distributed across the entire wetted width of the channel, so we chose three contiguous 20-m-long sections of river and divided them at the midline to create six replicate observation areas (mean width 5.6 m, mean area 112 m^2). In the lower reach, juvenile fish were restricted to the margins because of the greater thalweg velocities. We therefore chose three contiguous observation areas measuring 25 m long × 5 m wide along each stream edge for a total of six areas. To reduce the potential for the movement of fish in and out of study areas during the surveys, we located both study areas upstream of shallow riffles. The observation areas were marked during the first survey and were used in all subsequent surveys.

Surveys were initially conducted at 2- to 3-week intervals through the transition from summer to fall (August 22–23, September 14–15, September 27–28, October 19–20, 1995). One survey conducted in winter (February 28, 1996) and three more were done to complete the transition back to summer conditions (June 5, June 15, August 15–16, 1996). However, two of the surveys in the lower reach were abandoned because of ice cover (February 28) and poor water clarity (June 5). In each survey, we first collected data from the six observation areas of the upper reach over a single 24-h period: morning (08:00–12:00), afternoon (12:00 to 1 h before civil twilight), dusk (0.5 h before civil twilight to 0.5 h after civil twilight), and night (1–3 h after civil twilight). The following day, we repeated this protocol in the lower study. The exception was the first two surveys, when we collected data twice during each of the morning and afternoon periods. To collect observations, two divers simultaneously entered the study reach at its downstream boundary and moved slowly upstream counting fish in each replicate observation area. Counts were separated into groups corresponding to species and age-classes of fish present in the water column. We could not visually differentiate resident rainbow trout and anadromous steelhead trout juveniles, but based on the relative number of steelhead and rainbow trout spawning adults observed in the river, we think that most juveniles were steelhead trout. Thus, we refer to all *O. mykiss* as steelhead trout here. Trout were grouped into three age-classes based on size: fry (age 0), parr (age 1), and older (mostly age 2 but including a few to age 6+). We used hand-held lights in dusk and night surveys (Heggenes et al. 1993). Fish size was determined at the end of the growing season (October 8–14, 1996) using a backpack electrofisher to collect fish from each study reach. All fish that we captured were weighed (±0.1 g) and measured (±1 mm).

The highest counts were usually obtained in the dusk surveys, so we used the dusk data to estimate of the density of each species and age-class in the study areas. To standardize the fish counts, we calculated the ratio of the number of fish visible above the substrate in each swim to the number seen at dusk for that observation area. We report only steelhead trout and chinook salmon data, as we had too few observations of coho salmon to analyze meaningfully. We analyzed variation in the fraction observed relative to dusk with a split-plot analysis of variance (ANOVA) (Littell et al. 1991), with observation areas serving as subplots within reaches. We used the square root transformation to stabilize the variance of the data.

For surveys when all fish were concealed during the day, we also conducted predusk observations to determine when the first fish left its hiding place and became visible. Divers hovered over the study reach and recorded the time when the first fish was observed to leave the substrate.

To characterize the abundance of potential food organisms for juvenile salmonids, we sampled invertebrate drift concentration at the upstream boundary of each study reach on four dates in 1996. These data were collected as part of another research program; thus, the sample dates do not correspond to our snorkel survey trips. Six Mundie-type drift samplers (Mundie 1964) were installed across the upstream boundary of the study reach and were allowed to collect drifting invertebrates for 24 h. Invertebrates were removed from the samplers and placed in 3–5% buffered formalin for laboratory examination and enumeration. For this study, we report the total drift rate of all organisms enumerated as the number per square metre per second.

We tested a prediction of Clarke's (1994) asset protection principle, that smaller fish should be more likely to adopt a riskier diurnal activity pattern than larger fish. We estimated the size of fish that were active in the water column during the day and at night in late September, a time of the year when there was a mixture of activity patterns in the upper reach of the Bridge River in 1995. We predicted that smaller fish should be more likely to be in the water column during the day than larger ones so that the mean size of fish captured in the water column at night should be larger than those caught during the day. We captured age-0 steelhead trout and chinook salmon in the upper study reach at approximately 10:30, 13:30, 15:30, and 20:00 (dusk–night) on September 21, 1999. Water temperature increased from 11 to 14°C over the day. We captured fish that were active in the water column with two different

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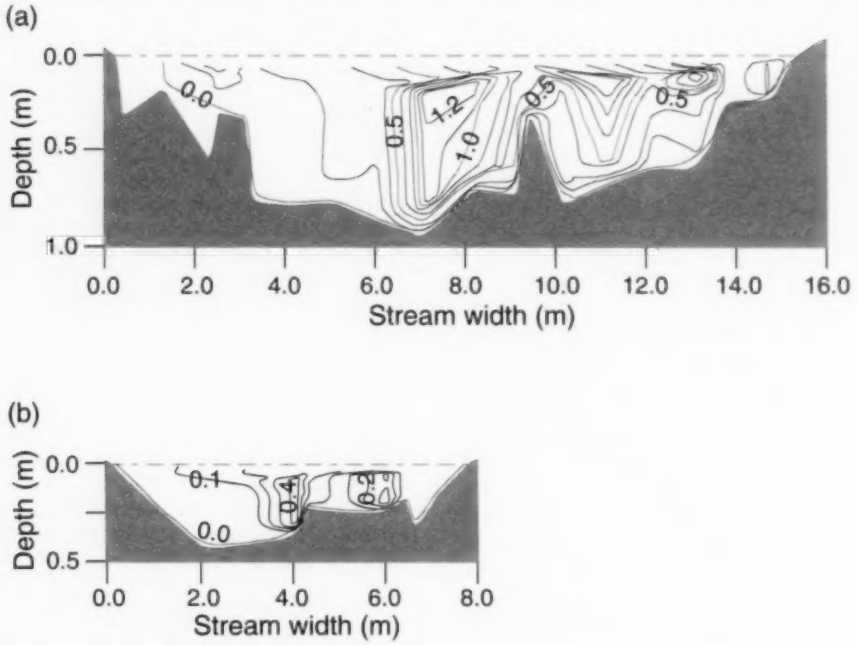
Fig. 2. Channel cross-section and velocity ($m \cdot s^{-1}$) isopleths for the (*a*) lower and (*b*) upper study reaches. Data are from a single transect located at the midpoint of each reach; the survey was conducted in October 1993 and is typical of the summer–fall low-flow period.



sampling techniques. First, we employed a diver who swam upstream with two small dip nets. The diver first located a fish in the water column and then used one net to drive it downstream into the capture net. Captured fish were then transferred to a bucket on the stream margin. We also used a pole seine and employed the diver to herd swimming fish into the bag of the seine. In both cases, we attempted to minimize disturbance of the area being sampled to prevent the dislodging of fish that might have been concealed in the substrate. We captured a total of 143 chinook salmon and 251 steelhead trout in the four samples. All captured fish were anaesthetized and their weight and lengths were measured; they were subsequently released just upstream of the study area. There is a chance that we may have recaptured some of the fish in later samples, although each sample consisted of a relatively small fraction of the fish that were present in the study area. We compared the mean size of captured fish from the four sampling periods with ANOVA that included gear type, time of day, and species as factors.

Results

The average density (as estimated by divers at dusk) of juvenile chinook salmon and steelhead trout in the summer and fall was 1.3 fish·m^{-2} in the upper reach and 0.23 fish·m^{-2} in the lower study area. Age-0 trout and chinook salmon were the most abundant, comprising 44 and 38% of the total, respectively. Age-0 trout were not encountered in the spring surveys because they were conducted before the cohort had emerged from their redds. Chinook salmon and steelhead trout fry were significantly larger in the upper reach than in the lower reach at the end of the growing season (Table 1); however, no difference was observed for trout parr.

There was considerable variation in the proportion of fish visible during the day relative to dusk (Figs. 3 and 4). We conducted an ANOVA on the afternoon data and found significant effects for fish category ($F_{3,257} = 20$, $P < 0.001$), reach ($F_{1,10} = 56$, $P < 0.001$), survey ($F_{6,257} = 13$, $P < 0.001$), and the interactions between fish category and reach ($F_{3,257} = 7.7$, $P < 0.001$), reach and survey ($F_{5,257} = 7.5$, $P < 0.001$), and fish category and survey ($F_{15,257} = 2.6$, $P < 0.001$). Winter data were excluded from this analysis because of many missing values.

The activity patterns of fish in the upper and lower reaches were very different. In the lower reach, nearly all juvenile salmonids were nocturnal in every survey (Fig. 3). During the day, fish were rarely observed above the substrate, but at dusk, we observed them leave their hiding locations and become visible to us. The first fish appeared at light levels of about 33 lm·m^{-2}, about 20 min before civil twilight. There was no relationship between light intensity at first emergence and water temperature ($r = 0.18$, $P = 0.7$, $n = 7$ surveys; temperatures ranged from 0 to 12.8°C). We observed that immediately after appearing above the substrate, juvenile salmonids held position in the current and appeared to be actively foraging on drift organisms. As light levels fell to nighttime levels, most fish moved to the margins of the river and were often resting on sand or silt substrates.

In the upper reach, activity patterns varied seasonally and among fish taxa (Fig. 4). In the winter, all fish were concealed, similar to what we observed in the lower reach. In the other seasons, fish were observed in the water column during the day. In general, the proportion of fish counted during the day relative to dusk was highest in the summer and was somewhat lower in the spring and fall surveys. The proportion of fish visible usually increased from morning to dusk. An exception was the summer data for age-0 trout (Fig. 4*a*), where relatively low dusk counts in one survey

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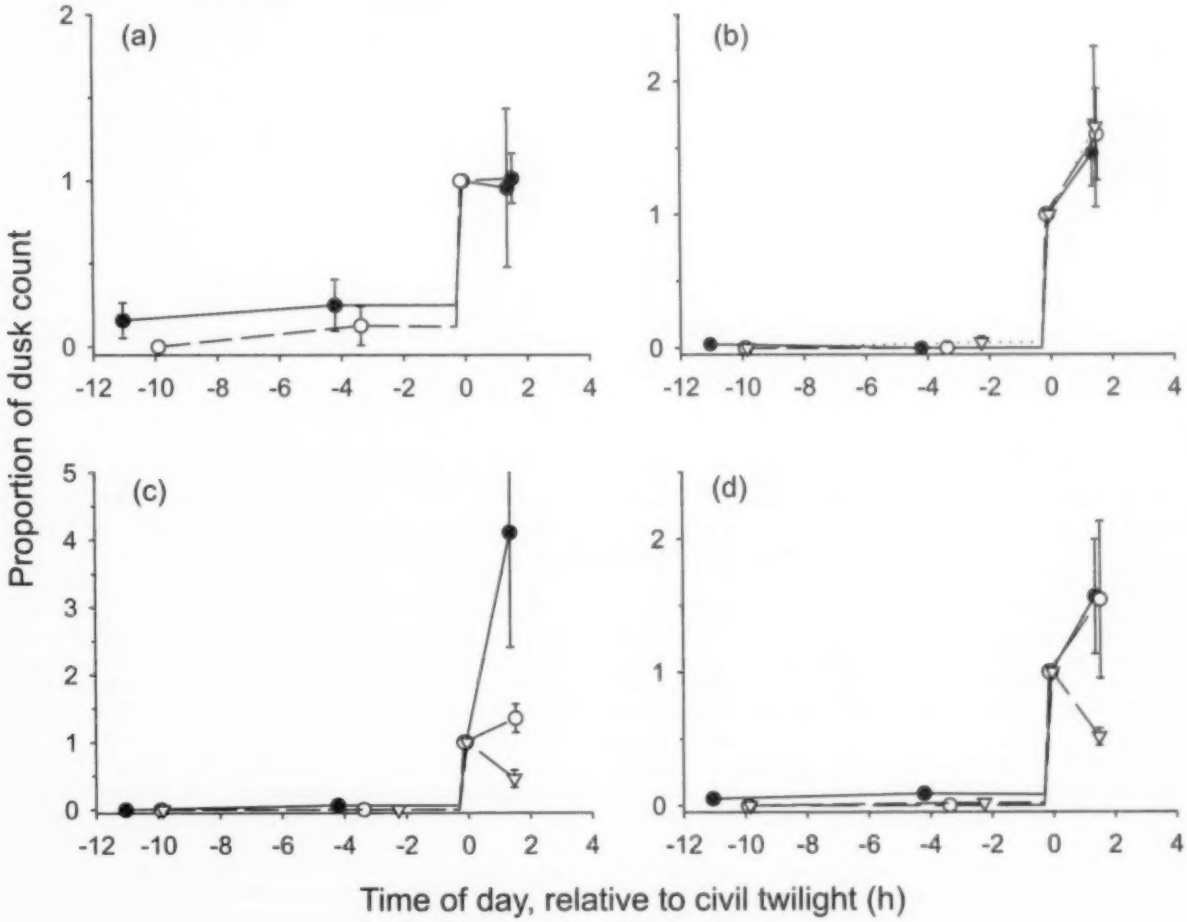
Table 1. Mean weight (g) of juvenile steelhead trout and chinook salmon in the upper and lower study reaches.

	Upper reach			Lower reach		
	Mean	SE	*n*	Mean	SE	*n*
Age-0 steelhead trout	2.6	0.1	753	2.0	0.1	242*
Age-1 steelhead trout	14.4	0.4	290	13.6	0.9	21
Age-2+ steelhead trout	46.0	6.1	10	—	—	—
Age-0 chinook salmon	4.5	0.1	490	4.1	0.05	162*

Note: Fish were sampled by electrofishing in mid-October 1996 as part of a larger assessment program. No age-2+ trout were caught in the lower reach. Asterisks indicate cases where there was a significant difference in weight between reaches (*t* tests, all $P < 0.01$).

Fig. 3. Mean proportion, relative to dusk counts, of (*a*) age-0 steelhead trout, (*b*) age-1 steelhead trout, (*c*) age-2+ steelhead trout, and (*d*) age-0 chinook salmon observed in the lower study reach (±SE, $n = 6$–12). Shown are averages for summer (solid circles), consisting of two August surveys, fall (open circles), consisting of three September and October surveys, and spring (triangles), consisting of two June surveys. The increase to the dusk value was set at 20 min before civil twilight, when fish were observed to begin leaving the substrate during falling light levels.



inflated the proportions for the day. Night counts were variable, partly because it was difficult to count fish that were resting on the bottom in shallows near the stream margins.

We found that the proportion of fish observed in the afternoon in the upper reach was correlated with water temperature (Fig. 5). In an analysis of covariance that included temperature, category of fish, reach, and site nested within reach as factors, the temperature term was significant ($F_{1,296} = 7.4$, $P = 0.005$) as was the interaction between temperature and reach ($F_{1,296} = 3.9$, $P = 0.05$) and the effect of fish category ($F_{3,296} = 22$, $P < 0.001$). Adjusted means from the analysis of covariance were used to compare fish categories; the

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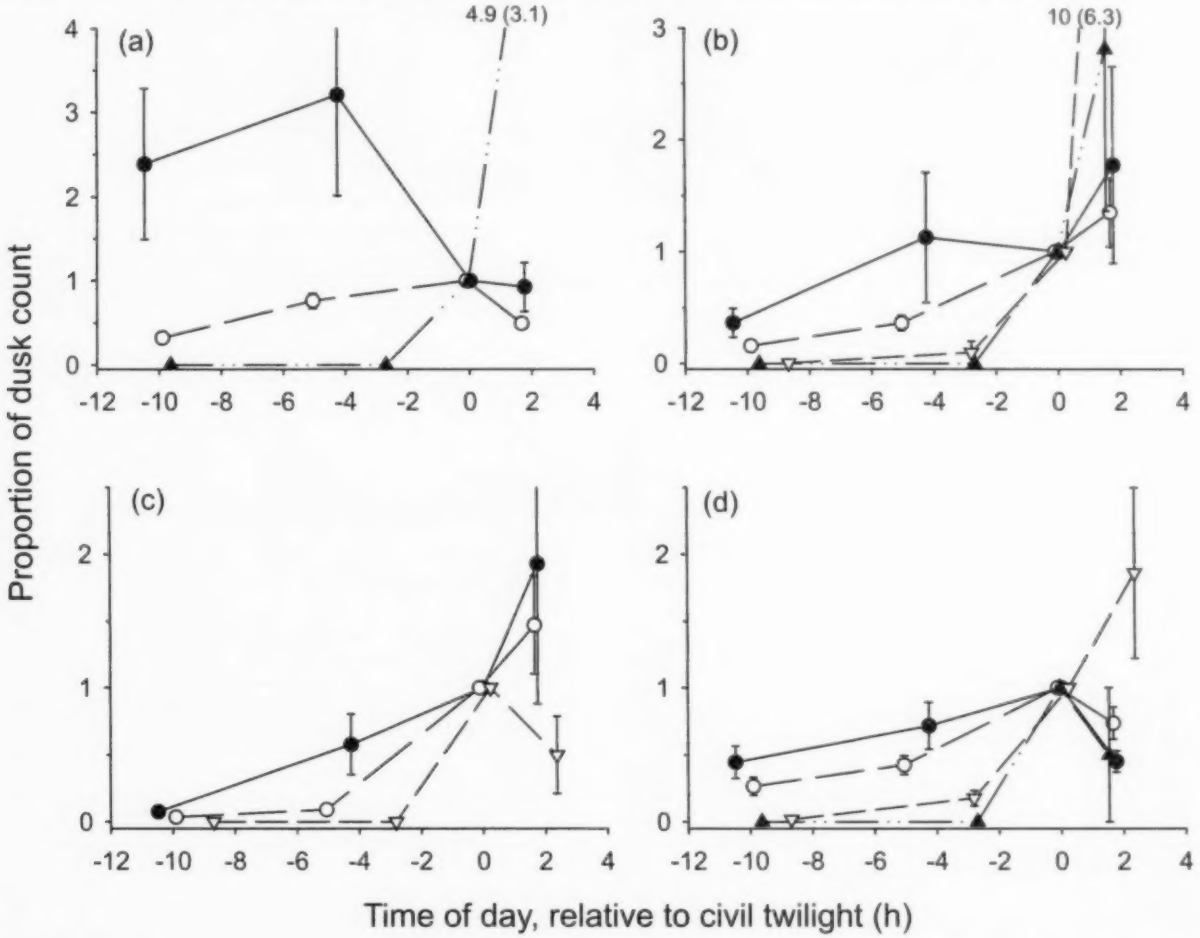
Fig. 4. Mean proportion, relative to dusk counts, of (*a*) age-0 steelhead trout, (*b*) age-1 steelhead trout, (*c*) age-2+ steelhead trout, and (*d*) age-0 chinook salmon observed in the upper study reach (±SE, n = 6–24). Shown are averages for summer (solid circles), consisting of two August surveys, fall (open circles), consisting of three September and October surveys, winter (solid triangles), consisting of a February survey, and spring (open triangles), consisting of two June surveys. Two off-scale points (with SE) are indicated.



results indicated that age-2+ trout were more nocturnal (P < 0.003) than chinook salmon and age-1 trout, which were similar to each other (P = 0.41). Steelhead trout fry were the most active in the afternoon compared with the other categories (P < 0.001). Thus, activity during the day was inversely related to body size.

Fish were observed in the water column in the upper reach at temperatures at which they were completely concealed in the lower area (Fig. 5). The upper reach was usually warmer than the lower area (Fig. 1) so that comparisons based on temperature alone are potentially confounded by seasonal effects. However, there was one survey in late September 1995 in which the difference between reaches was <1°C; these data allow a test of the effect of reach, independent of season and temperature. Results from an ANOVA of the data from this survey indicate that a significantly higher proportion of fish were observed in the upper reach compared with the lower one ($F_{1,10}$ = 36, P < 0.001) (Fig. 5, symbols connected by lines).

We did not find any consistent differences in the estimated rate of invertebrate drift between the study reaches (Table 2). In the ANOVA of log-transformed data, none of the sources of variation were statistically significant (month: $F_{3,40}$ = 2.4, P = 0.08; reach: $F_{1,40}$ = 1.6, P = 0.21; month × reach: $F_{3,40}$ = 2.2, P = 0.11).

In our test of the asset protection principle, we found that fish captured during the day were significantly smaller than those caught at dusk and night (Table 3; Fig. 6). However, there was considerable overlap in fish size between the daylight and night samples (Fig. 6). Juvenile chinook salmon taken by the dip net were consistently larger than those captured by seine; this resulted in a significant gear and gear × species effect in the ANOVA results (Table 3).

Discussion

Our study provides one of the most complete set of observations on seasonal and spatial variation in activity patterns

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Fig. 5. Relationship between the mean proportion of (*a*) age-0 steelhead trout, (*b*) age-1 steelhead trout, (*c*) age-2+ steelhead trout, and (*d*) age-0 chinook salmon active during the afternoon observation period relative to dusk and river water temperature in the upper (solid circles) and lower (open circles) study reaches (±SE, n = 6 –12). Each symbol represents a different survey. Lines connect a late September survey conducted when the difference in water temperatures between reaches was <1°C. One off-scale point (with SE) is indicated.

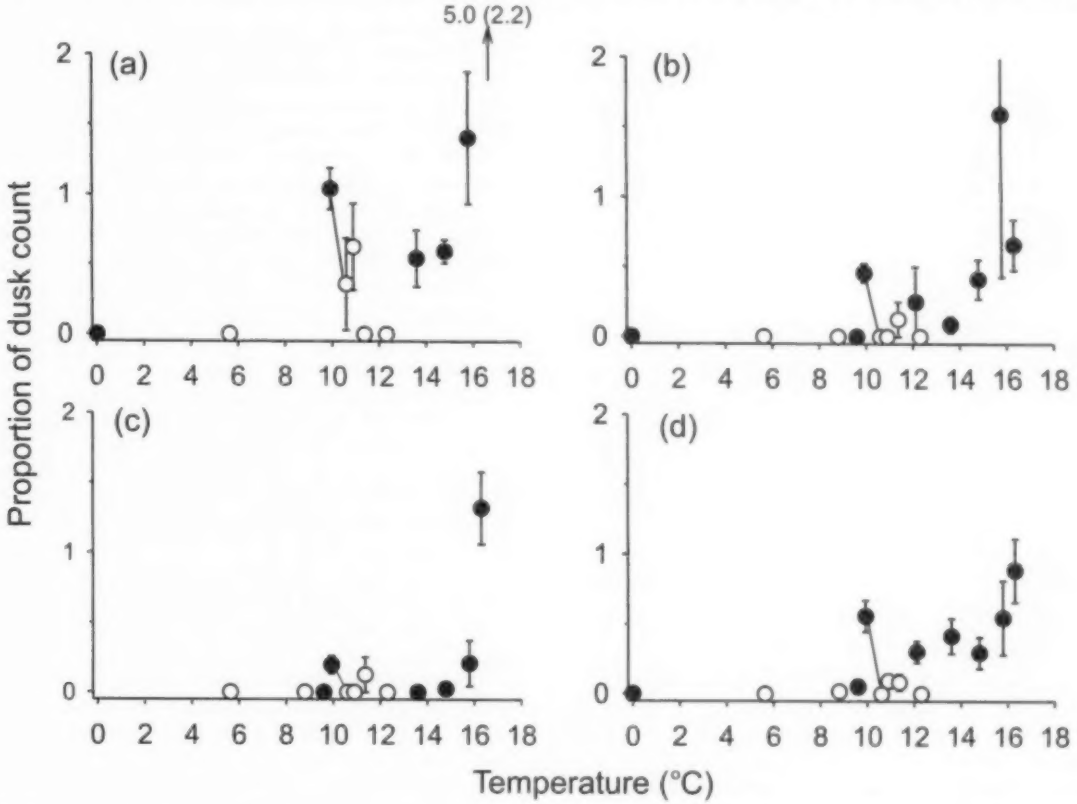

Table 2. Summary of estimates of the invertebrate drift rate (organisms·m^{-2}·s^{-1}) from the Bridge River.

	Upper reach		Lower reach	
Date (1996)	Mean	SE	Mean	SE
June 19–20	1.9	0.2	2.3	0.3
August 16–17	2.0	0.3	3.3	0.5
September 26–27	2.2	0.8	3.3	0.5
October 10–11	6.1	1.9	3.0	0.4

Note: Statistics are based on six 24-h samples collected in each reach.

Table 3. ANOVA statistics for the test of the relationship between the length or weight of age-0 steelhead trout and chinook salmon and the time of day of capture (morning, early or late afternoon, dusk–night) or gear type used (dipnet or pole seine).

		Length		Weight	
Source	df	F	P	F	P
Species	1	949	<0.001	827	<0.001
Time	3	5.0	0.002	4.5	0.004
Gear	1	9.6	0.002	21.2	<0.001
Species × time	1	0.4	0.75	1.5	0.23
Time × gear	3	0.6	0.60	0.3	0.81
Species × gear	1	12	0.006	25	<0.001
Species × time × gear	3	0.2	0.9	0.2	0.87
Dusk vs. day	1	9.8	0.002	4.4	0.037

Note: The length data are plotted in Fig. 6. The last row refers to a contrast of the dusk sample with the other three samples taken during the day. In all cases, the error df = 378.

and is the first to demonstrate that within one river, there can be variation among individuals of the population, depending on local habitat conditions. Our results extend a growing body of evidence that stream salmonids can display quite complex patterns of activity (Campbell and Neuner 1985; Fraser et al. 1995; Gries et al. 1997) and corroborate the hypothesis that diel activity patterns are the result of interactions between individuals and their environment.

Habitat-specific activity patterns

The contrast in the observations between the upper and lower reaches supports the view that activity patterns are due to differences in habitat and not simple functions of temperature or photoperiod. We found that some age-0 steelhead trout and chinook salmon were day active in the upper reach at temperatures at which they were completely concealed the lower reach. Thus, while temperature is an important factor

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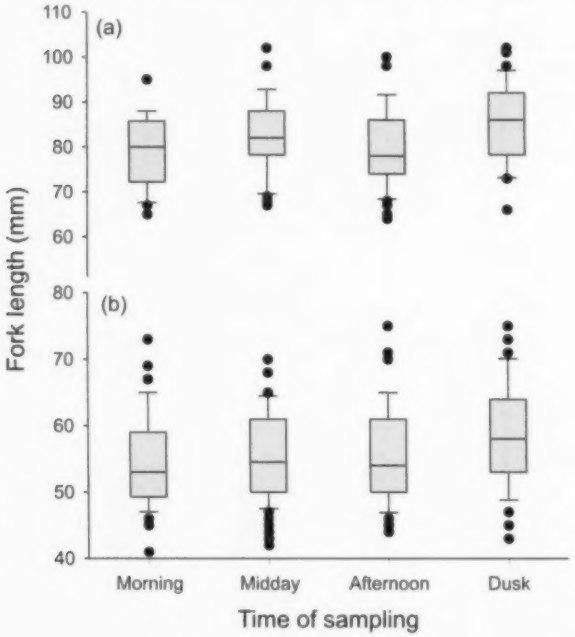
Fig. 6. Box plots for the length of (*a*) age-0 chinook salmon and (*b*) steelhead trout captured by pole seine and dip net in the water column in the upper study reach on September 21, 1999. The boxes define the 25th and 75th percentiles; the error bars indicate the 5th and 95th percentiles. Samples sizes range from 27 to 70.



causing variation in behaviour within a habitat, in the Bridge River, it did not explain differences between the study reaches.

Can the differences in activity between the reaches be explained in terms of the growth – risk of predation trade-off? Theory suggests that in the lower reach, either the foraging conditions were better or the risk of daytime predation was higher, causing virtually all fish to be crepuscular or nocturnal. Our drift data did not indicate that the 24-h drift rates were consistently higher in the lower reach, but the nature of our data means that we cannot discount the possibility that there were differences in the diel cycles of drift. If there was a large increase in drift during the crepuscular period in the lower reach (Rader 1997), then fish may have been able to obtain adequate ration during short feeding bouts at dusk or dawn. Juvenile salmonids were also much less dense in the lower reach, which may have improved the foraging success if there was competition for drift or foraging locations. Quantitative information on the abundance of predators are lacking, but we observed that large bull trout are found only in the lower reach, probably because of the presence of deeper water, larger pools, and lower summer water temperatures (Rieman and McIntyre 1993).

Could daytime activity in the upper reach be explained by competition for limited hiding spaces relative to the density of fish? Smaller fish can be at a disadvantage in finding hiding spaces at dawn relative to larger ones (Gregory and Griffith 1996). The substrate of the upper study reach consists of uncompacted cobble and boulder because sediment input is limited by the dam, and spills from the dam in 1991 and 1992 (Higgins and Bradford 1996) were sufficient to mobilize the streambed and remove sediment. Thus, although the average density of all juvenile salmonids was relatively high in the summer and fall, we believe that the density of hiding places exceeded the density of fish. Competition for hiding places also does not explain the trend towards increased activity over the day in the upper reach. Daily and seasonal variation in the proportion observed during the day suggests that activity patterns are more a function of biological factors than of the physical characteristics of the streambed.

Trout and chinook fry were smaller in the lower reach, which may have been the result of lower water temperatures there. Presumably, fish in the lower reach could have increased their growth by foraging during the day. We can only surmise that the benefits of additional foraging during the day may have been outweighed by the risks of predation in the lower reach.

Variation within the upper reach

In the winter survey, all fish were nocturnal in the upper reach, similar to what has been observed in many other studies (e.g., Campbell and Neuner 1985). Although the feeding efficiency of juveniles is sharply reduced at night, metabolic rates are very slow, and fish may be able to obtain an adequate energy intake by feeding between dusk and dawn (Fraser and Metcalfe 1997).

The proportion of fish observed in the water column increased over the day in most of the summer and fall surveys in the upper reach (Gries et al. 1997). We do not know whether fish leave the substrate, feed, and return or whether we are seeing fish leave the substrate and stay in the water column for the remainder of the day. We speculate that fish activity may be partially motivated by hunger, which may cause more fish to forage in the afternoon as stomach fullness resulting from feeding the previous night declines (Metcalfe et al. 1999). This effect will be greatest in the summer because days are longer and temperatures are higher, resulting in a more complete digestion of food obtained during the previous night. Hunger has been shown in a number of studies to affect foraging decisions under the risk of predation (Gotceitas and Godin 1991; Damsgård and Dill 1998).

In the upper reach, we found that larger species or age-classes were more nocturnal than smaller ones, which is consistent with the results of some other field studies (Hillman et al. 1992; Gries et al. 1997; but see Gries and Juanes 1998). Our data also suggest that the relationship between activity and temperature may also vary with fish size. Since metabolic and digestive rates, relative meal sizes, and the scope for growth decline with increasing body size (Brett and Groves 1979), larger fish may be able to obtain an adequate ration with a largely crepuscular foraging regime and thus are more nocturnal at a given temperature than smaller fish. Conversely, smaller age-classes may be more active earlier in the day because their higher metabolic rate results in the faster digestion of food obtained during crepuscular or nighttime feeding (Krause et al. 1998).

We were able to find weak, but not particularly compelling, support for the asset protection principle. The principle predicts that there should be a threshold size for nocturnalism and that individuals smaller than the threshold should

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assume a diurnal strategy to increase their growth rate (Clarke 1994). We purposely sampled at a time of year when nocturnal and diurnal fish were both present, and there was a wide range in body size for each taxon (the steelhead trout fry that we captured ranged from 0.8 to 6.6 g; for chinook salmon, the range was 3.1–13.8 g). While we found a very slight trend towards a larger mean size with time of day, the range in size between the daytime and nighttime samples was similar. We might have expected to see more large fish in the night samples, if all large fish had been adopting the risk-adverse sheltering behaviour until dusk.

The very small difference in the mean size of day-active versus nocturnal fish suggests that variation in behaviour might be dominated by short-term decisions about activity rather than state-dependent behaviour related to a relationship between body size and fitness at some time in the future. The predictions of the asset protection principle are contingent on the satisfaction of the assumption that there is a relationship between size at a certain time and subsequent survival or fitness (Houston et al. 1993). If size and survival are independent, then individuals should adopt the behaviour than minimizes u/g each day, regardless of their size. For stream-dwelling salmonids, survival is not always a function of size (Meyer and Griffith 1997), which might be why our data for the Bridge River do not seem to support Clarke's (1994) model. Factors unrelated to fish size such as recent foraging success, exposure to predators, and possibly social interactions could be contributing factors influencing daily decisions about foraging activity (Damsgård and Dill 1998).

Implications of variation in diel activity patterns

The productivity of salmonid populations residing in shallow streams has been described as being influenced primarily by limits imposed by their territorial behaviour. As fish grow, their territories increase in size, and there is usually a corresponding decrease in fish density (Grant and Kramer 1990). Our results show that territorial models for population regulation may need to consider the daily dynamics of activity and concealment (Gries et al. 1997). For example, in the lower reach of the Bridge River, fish forage briefly at dusk (and perhaps dawn), and we do not know if fish form brief territories or dominance hierarchies during the feeding periods. If that was the case, these would have to be formed twice daily at dusk and dawn, as fish either descend into the substrate during the day or rest in shallow areas at night. In the upper reach, some fish may be involved in territory defense (especially in the summer afternoons), but this is abandoned after dusk. For most of the year, many fish remain concealed in the substrate during daylight hours. These complex behaviours may invalidate simple territory size models for estimating stream capacity.

Similarly, analyses that attempt to predict salmonid abundance or the effects of ecosystem changes (particularly flow) from physical habitat measurements should also incorporate information on activity patterns. Often, a model that links physical habitat and habitat preferences of the fish would be used to predict the effects of different flows on fish populations (Jowett 1997). The main physical difference the upper and lower reaches of the Bridge River is flow, but we do not know whether variation in diel activity patterns between reaches is related directly to flow or to some other habitat factor. Current models cannot predict whether activity patterns will change with flow, and we do not understand how productivity of the population might be related to changes in activity. Active experimentation may be the only way to understand the relationship between flow and fish populations when there is such complexity at the level of the individual (Walters 1997).

In summary, we found a variety of activity patterns among juvenile salmonids in the Bridge River, which seem to be the result of relatively fine-scale effects of habitat on individual fish. Clearly, more work in natural settings is required to understand these behaviours. If such patterns are widespread, we may need to think more about how variation in behaviour among individuals could affect salmonid production in small streams.

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Detection of population trends in threatened coho salmon (*Oncorhynchus kisutch*)

Katriona Shea and Marc Mangel

Abstract: Populations of coho salmon (*Oncorhynchus kisutch*) in California are listed as threatened under the U.S. Endangered Species Act. Such listings refer to adult populations, but often, juvenile life history stages are censused, so it is important to understand what affects the relationship between true adult and observed juvenile numbers. We present models to address how observational uncertainty, census length, and autocorrelation in vital rates affect our ability to observe trends. We ask two questions about our ability to detect declines in one life history stage from censuses of another. First, given an observed decline in parr numbers, what is the chance that this reflects a decline in adults? Second, given that adult numbers are declining, what is the chance that we see that decline in parr? Our results indicate that statistical power decreases with increasing observational uncertainty and decreasing census lengths and demonstrate how these two parameters interact. Power increases as the level of autocorrelation in mortality rates increases. Management recommendations include obtaining more accurate estimates of autocorrelation in mortality and of observational uncertainty.

Résumé : Les populations du Saumon coho (*Onchorhynchus kisutch*) de la Californie apparaissent sur la liste des espèces vulnérables conformément à la loi américaine sur les espèces menacées. Cette désignation concerne les populations d'adultes; comme il y a souvent des stades plus jeunes qui sont inclus dans les inventaires, il importe de comprendre les détails de la relation qui unit les nombres des vrais adultes et les nombres observés de jeunes. Des modèles permettent de comprendre comment l'incertitude des observations, la durée des inventaires et l'autocorrélation dans les taux vitaux affectent notre habileté à observer les tendances. Nous posons deux questions sur la possibilité de détecter les déclins dans un stade du cycle biologique à partir d'inventaires faits sur un autre stade. Premièrement, quelle est la probabilité d'avoir un déclin dans la densité des adultes lorsqu'on observe une diminution des densités des tacons? Puis, quelle est la probabilité de discerner chez les tacons un déclin qui se produit chez les adultes? Nos résultats montrent que la puissance statistique diminue lorsque l'incertitude des observations augmente et la durée des inventaires diminue; ils illustrent aussi l'interaction entre les deux variables. La puissance croît à mesure que l'autocorrélation entre les taux de mortalité augmente. Pour fins d'aménagement, nous recommandons d'obtenir des estimations plus précises de l'autocorrélation de la mortalité, ainsi que de l'incertitude des observations.

[Traduit par la Rédaction]

Introduction

Several of the seven Pacific salmon species, including coho salmon (*Oncorhynchus kisutch*), have recently been listed as threatened under the U.S. Endangered Species Act. Californian populations of this species have declined considerably in the past century (Brown et al. 1994). In response to this decline, distinct populations of coho in California were defined and listed in terms of evolutionary significant units (National Marine Fisheries Service 1995; Weitkamp et al. 1995). Coho in the central California evolutionary significant units were listed in October 1996 and in the southern Oregon – northern California evolutionary significant units in May 1997 (Spencer 1999).

Status reviews for this species in Washington and Oregon focus on spawner time series, which extend back to the 1950s (Weitkamp et al. 1995). In California, however, data are far less extensive (Brown et al. 1994; Weitkamp et al. 1995). Much of the focus is on presence–absence data, and few ongoing field surveys were commenced before 1990 (Spencer 1999). Furthermore, these surveys have mainly focused on river-dwelling juvenile stages (parr) and include snorkel surveys of juvenile numbers and electrofishing (P.B. Adams, National Marine Fisheries Service, Santa Cruz, CA 95064, U.S.A., personal communication). As U.S. Endangered Species Act listing and delisting assessments usually pertain to adult numbers (in the case of salmon, spawner escapement), it is necessary for there to be an understanding of the relationship between adult and juvenile numbers. Given that the endogenous and exogenous factors affecting the life history of this species are uncertain and that sampling efforts introduce additional uncertainty, this is not a trivial link to evaluate.

Uncertainty in this system arises from three main sources (Hilborn and Mangel 1997). Some of this uncertainty is endogenous, including uncertainty about biological processes such as mortality (particularly in the ocean-dwelling phase)

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Table 1. Life history of coho salmon.

Year	Season	Event
t	Autumn	Spawning occurs and eggs are laid
$t + 1$	Spring	Parr emerge
$t + 2$	Spring	Smolts migrate
$t + 2$	Autumn	Jacks return (male only) (zero sea winters: 6 months)
$t + 3$	Autumn	Adults return (male and females) (one sea winter: 18 months)
$t + 4$	Autumn	Adults return (females only) (two sea winters: 30 months)

Note: For most of the range of coho, spring indicates April or May, while autumn indicates November. In California, the timing of events tends to be later in the year (Shapovalov and Taft 1954).

and demographic and genetic stochasticity. There are also exogenous sources of uncertainty: the amount of, and role played by, environmental stochasticity and catastrophes. Finally, there is observational uncertainty, arising from sampling efforts. This uncertainty limits our ability to detect changes in population levels and hampers management efforts.

Development of models for this system involves summarizing known biological and ecological aspects and hence will highlight important unknown quantities. Models will allow us to target which aspects are most important to understand fully, using different types of sensitivity analyses. Once developed, such models can also be used to direct empirical research efforts, to focus on management strategies that seem most likely to succeed in achieving conservation goals, and to ask ecological and management questions that are hard, or even impossible, to answer in the field.

We first present a model that summarizes the most general biology and life history of this species. This generic model comprises a series of nested, subsidiary models. Using this framework, we can compare models of increasing complexity (incorporating different life history and environmental factors) under different levels of observational uncertainty. This will highlight which parts of the life history it is important to know best (a target for future experimental research) and also whether existing sampling methodologies are sufficiently accurate to be useful in this system.

Sensitivity analyses allow us to estimate the relative "value" (i.e., usefulness) of different types of information for this system. Hence, we can ask which data are worth spending time and resources to obtain and which information we can manage without. Thus, our objective is to make recommendations to managers, not for management. Sensitivity analyses can be carried out at one of three levels. Sensitivity can be examined at the level of the model structure (e.g., by including or excluding different life history stages), at the level of the mathematical form (e.g., ± environmental autocorrelation), or at the level of the parameter (e.g., differing amounts of autocorrelation). Some, or all, of these different types of analyses can be carried out for all factors about which there is uncertainty.

We then present a simplified life history model for coho. We use this specific model to assess the power that current sampling methods for juveniles (parr) have to tell us about trends in adult populations. We ask how observational uncertainty, length of time series, and autocorrelation in vital rates affect our ability to observe the true state of affairs. We pose two main questions. First, given an observed decline in parr numbers, what is the chance that this reflects a decline in adults? Second, given that adult numbers are declining, what is the chance that we see that decline? We assess how the answers to these questions change in response to changes in observational uncertainty, census length, and autocorrelation in vital rates. The relative importance of these factors will highlight whether existing sampling methodologies are sufficiently accurate to be useful in this system.

Methods

Summary of coho salmon biology, life history, and ecology

Coho salmon is one of the seven Pacific salmon species (genus *Oncorhynchus*). Coho have a semelparous life history strategy (Shapovalov and Taft 1954; Sandercock 1991). Late in the year, adults migrate from the ocean to their natal streams, where spawning occurs. After spawning, they die. Parr emerge the next spring and usually remain in freshwater for 1 year before migrating to the sea as smolts. The majority of these fish remain in the ocean for 18 months before returning to freshwater to begin the cycle again. Some males, called jacks, return after only 6 months at sea. Some females may remain at sea for two winters, returning to spawn after 30 months. A summary of this cycle is shown in Table 1. For further details, see Sandercock (1991).

Coho salmon life history model

Figure 1*a* shows a life cycle diagram for coho salmon. A simplified version of this life cycle, in which the early-returning jacks and late-returning females (which form a minority of the population) are ignored, is presented in Fig. 1*b*. The model equations for the simplified life history are presented in the Appendix.

The model is a discrete-time, age-structured model, with time steps of 1 year. Censuses are modeled on an annual basis with a defining cutoff in January (i.e., after spawning and before emergence and migration). The number of individuals (both male and female) in a life history stage is considered either in summer and early autumn (juveniles) or in late autumn (adults) of any given year. Note that an egg stage is not included explicitly, due to the model timing, but that data on egg production are included in the parr stage formula. We assume a 50:50 sex ratio. We do not include density dependence in this version of the model, since populations of this species have been dramatically reduced from historical levels.

Observational uncertainty

Observational uncertainty in the models is assumed to follow a lognormal distribution, where X is $N(0,1)$ and σ_{obs} is the standard deviation of the observational uncertainty (see Hilborn and Mangel 1997, p. 146, 7.33). Thus, observed parr, P_{obs}, are related to true parr, P, by

$$(1) \qquad P_{obs} = Pe^{(\sigma_{obs}X - \frac{1}{2}\sigma_{obs}^2)}.$$

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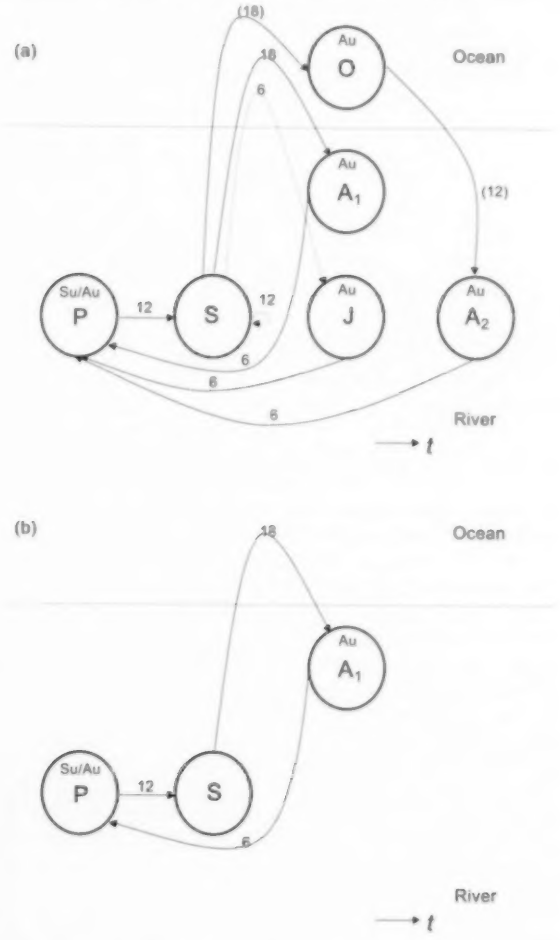
Fig. 1. (*a*) Full and (*b*) simplified life cycle diagrams for coho salmon. Circles represent life history stages of the coho. P, S, O, A_1, A_2, and J refer to the densities of parr, smolts, ocean-dwelling salmon, one-sea-winter adults, two-sea-winter adults, and jacks, respectively, all at time t. Arrows represent transitions between these stages. Time t (in years) can be read from left to right, as indicated. Stages or transitions below the dotted line occur in the river. Stages and transitions above the line occur in the ocean. Su/Au or Au at the top of a stage indicates that that stage is counted in late summer – early autumn or in autumn, respectively. The number above any transition indicates the time (in months) between the two linked life history stages.



We used the Box–Muller method (Press et al. 1989) to generate normal random variables.

Detection of trends in abundance over time

This work concerns our ability to correctly detect trends in abundance. Type I errors occur when there is no trend in abundance, but we incorrectly conclude that one exists. Type II errors occur when there is a trend in abundance, but we falsely conclude that none exists. If the probability of making a Type II error is β, then the statistical power is $1 - \beta$.

Statistical power to detect trends in abundance (Gerodette 1987; Peterman and Bradford 1987; Peterman 1990) is especially vital in endangered populations. In the present paper, we are interested in the statistical power to detect trends in true adult numbers from observed trends in juvenile numbers. In order to assess this, we examined how adult and juvenile numbers changed through time and how these trends were correlated.

Population trajectories of 300 years were simulated using the specific model. All analyses were carried out after at least 90 years to ensure that transients had disappeared. Regression analyses were based on subsets of the full trajectory of 5-, 10-, 15-, or 20-year observation windows. For each trajectory, regression analyses of abundance through time were carried out for each of three variables: true adult numbers, $A(t-1)$, true parr numbers $P(t)$, and observed parr numbers $P_{obs}(t)$. In each case, a regression slope was calculated and tested against the null hypothesis that the slope was not significantly different from zero (i.e., the population size is staying the same) in a two-tailed t test at the 5% level (Sokal and Rohlf 1995). If the slope was significantly different from zero, the sign of the regression coefficient indicated whether the trend was positive or negative. We also carried out a parallel analysis for a scenario with the burden of proof placed on showing that there is a trend. Such analyses have been called for in fisheries management (Peterman 1990) and clearly would be of use in conservation situations also. In this case, we tested for significant declines or increases using two one-tailed t tests, again at the 5% level (thus, in effect, we tested overall for a 10% chance of a change in population size). All these analyses were repeated 1000 times for each combination of four lengths of observation window (w = 5, 10, 15, and 20 (at least 3 years of data are required for a complete life cycle to be observed)) over all values of year-to-year autocorrelation in mortality, ρ (from 0 to 1 in increments of 0.05), and over all values of observational uncertainty, σ_{obs} (from 0 to 2 in increments of 0.05). The results of the 1000 runs for each parameter combination were used to calculate the probabilities of the different possible outcomes.

For any parameter set, we can then see how trends in one variable (e.g., true adult numbers) related to trends in any other variable (e.g., observed parr numbers). This allows the estimation of a measure of the power that we have to assess population trajectories correctly. There are two main questions of interest for the simulated population.

Question 1. Given that we see a decline in parr numbers, what is the chance that this reflects a decline in adults?

We let $\Pr\{A\downarrow\}$ denote the probability that the true adult population is declining and $\Pr\{P_{obs}\downarrow\}$ the probability that the observed parr population is declining. The question that we are asking concerns the conditional probability that true adult numbers are declining, given that observed parr are declining, $\Pr\{A\downarrow|P_{obs}\downarrow\}$. Using the standard definition of conditional probability, we obtain

$$(2) \qquad \Pr\{A\downarrow|P_{obs}\downarrow\} = \Pr\{P_{obs}\downarrow, A\downarrow\}/\Pr\{P_{obs}\downarrow\}.$$

The complement of this probability, $1 - \Pr\{A\downarrow|P_{obs}\downarrow\}$, lets us ask "when adults are not declining, what is the chance that we mistakenly see that it is, i.e., that we see juveniles declining?" If we are wrong in answering these questions, we are making a Type I error.

Question 2. Given that adult numbers are declining, what is the chance that we see a decline in parr?

This question asks what is the conditional probability that observed parr are declining, given true adults are declining, $\Pr\{P_{obs}\downarrow|A\downarrow\}$?

$$(3) \qquad \Pr\{P_{obs}\downarrow|A\downarrow\} = \Pr\{P_{obs}\downarrow, A\downarrow\}/\Pr\{A\downarrow\}.$$

If we are wrong, then we are making a Type II error.

The results for both these questions can be presented in a probability tree (Fig. 2), following the method of Peterman and Bradford (1987). These two results are linked by Bayes' theorem:

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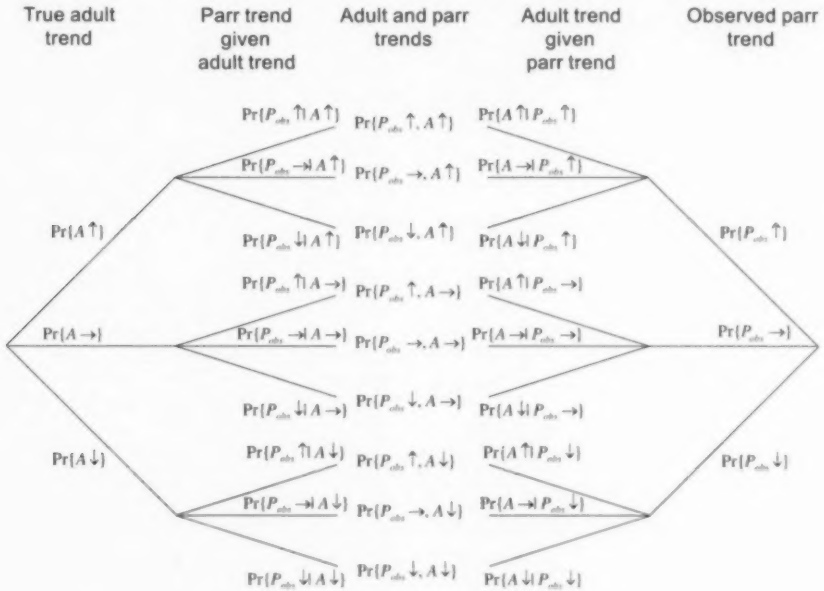
Fig. 2. Possibility tree for the trends in coho salmon true adult population and observed parr population sizes. Population trajectories for each can either increase (↑), remain the same (→), or decline (↓). Thus, for example, $\Pr\{A\downarrow\}$ denotes the probability of a decline in true adult numbers, $\Pr\{P_{obs}\rightarrow\}$ denotes the probability of the observed parr population size remaining constant, $\Pr\{A\uparrow, P_{obs}\uparrow\}$ denotes the probability that observed parr and true adult populations both increase, and $\Pr\{P_{obs}\downarrow|A\downarrow\}$ denotes the probability that we observe a decline in parr given a decline in the true adult population. See Fig. 3 for a specific example.



(4) $\Pr\{A\downarrow|P_{obs}\downarrow\} = \Pr\{P_{obs}\downarrow|A\downarrow\}\Pr\{A\downarrow\}/\Pr\{P_{obs}\downarrow\}.$

An example of the calculation of the conditional probabilities $\Pr\{A\downarrow|P_{obs}\downarrow\}$ and $\Pr\{P_{obs}\downarrow|A\downarrow\}$ is shown in Fig. 3 for the case where the window of observation $w = 20$, the autocorrelation in mortalities $\rho = 0.5$, and the observation uncertainty $\sigma_{obs} = 1.0$. From this tree, it can be seen that

(5) $$\Pr\{P_{obs}\downarrow|A\downarrow\} = \Pr\{P_{obs}\downarrow, A\downarrow\}/\Pr\{A\downarrow\} = 0.096/0.196 = 0.490$$

and

(6) $$\Pr\{A\downarrow|P_{obs}\downarrow\} = \Pr\{P_{obs}\downarrow, A\downarrow\}/\Pr\{P_{obs}\downarrow\} = 0.096/0.112 = 0.857.$$

Note that there are two sources of uncertainty in the relationship between true adult numbers and observed parr. First, there is the relationship between observed juvenile numbers and actual juvenile numbers. Second, there is the relationship between actual juvenile numbers and actual adult numbers. Thus, these questions involve the true parr number as a hidden state variable.

Results

The ability to correctly discern the relationship between observed parr and actual adult number, as defined by $\Pr\{A\downarrow|P_{obs}\downarrow\}$ and $\Pr\{P_{obs}\downarrow|A\downarrow\}$, depends on the number of years for which surveys are carried out (the "window"), w, on the amount of autocorrelation in stage mortalities from one year to the next, ρ, and on the observational uncertainty, σ_{obs} (Figs. 4 and 5). Note that all of the uncertainty in the relationship between true adult numbers and observed parr numbers in this model was due to the uncertainty in the relationship between actual and observed parr numbers, i.e., due to observational rather than biological uncertainty. True adult and true parr numbers always (i.e., for all simulations, for all w, ρ, and σ_{obs}) showed the same trends (↑, →, ↓).

In general, as σ_{obs} increases, $\Pr\{P_{obs}\downarrow|A\downarrow\}$ and $\Pr\{A\downarrow|P_{obs}\downarrow\}$ tend to decrease. Figure 4 shows these results for fixed ρ and different values of w. As the observational uncertainty increases, it becomes increasingly difficult to see the true state of affairs. As w increases, both $\Pr\{P_{obs}\downarrow|A\downarrow\}$ and $\Pr\{A\downarrow|P_{obs}\downarrow\}$ increase (Figs. 4 and 5), and variation in these estimates decreases. Thus, longer census windows increase our ability to see clearly what is going on in the system. Finally, as ρ increases, $\Pr\{P_{obs}\downarrow|A\downarrow\}$ and $\Pr\{A\downarrow|P_{obs}\downarrow\}$ both tend to increase. Figure 5 shows these results for fixed σ_{obs} and different values of w. Increased correlation in mortality rates improves our ability to correctly detect trends in population size.

The result for ρ is less obvious than those obtained for σ_{obs} and w and deserves further examination. If autocorrelation is low or zero, then mortalities can quickly vary from high to low between years. If, however, autocorrelation is high, mortalities will tend to remain the same for long periods of time, i.e., a trend is set up. If, for example, mortality is high in one year, it is likely to remain high for several years in a row. Consistent high mortality rates will drive the population size down, and we are more likely to see a strong decline. Similarly, with consistent low mortality rates, we are more likely to observe an increase in population size than we would in the absence of autocorrelation. Closer examination of the probability of generating a decline in true

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Fig. 3. Probability tree illustrating how the general method is applied (window of observation $w = 20$, autocorrelation in mortalities $\rho = 0.5$, observation uncertainty $\sigma_{obs} = 1.0$). Note that the probabilities in each of the three columns sum to unity.

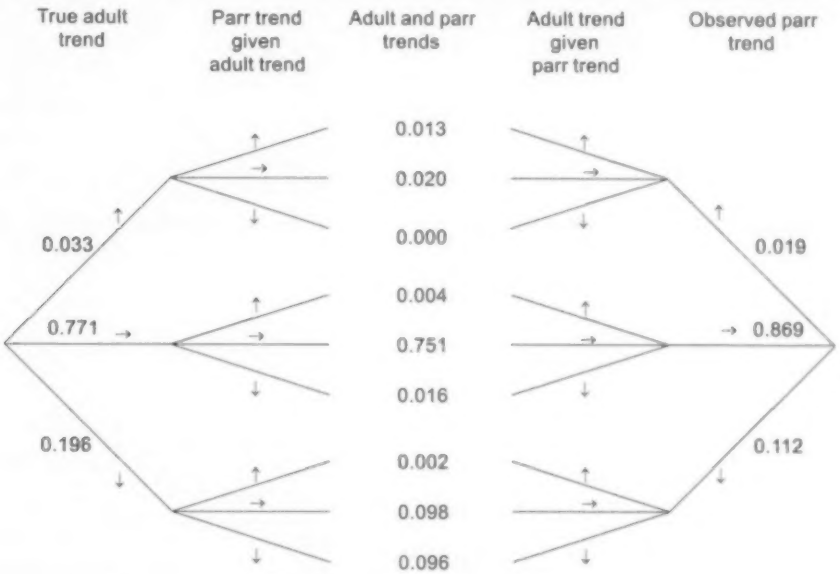

Table 2. Conditional probabilities for $\sigma_{obs} = 0.4$ (estimated from marine survival data; Berman 1999) and $\rho = 0.29$ (estimated from coho data from the Oregon Production Area).

Census window,	Change in size*		Increase or decrease in size†	
w (years)	$\Pr\{P_{obs}\downarrow \mid A\downarrow\}$	$\Pr\{A\downarrow \mid P_{obs}\downarrow\}$	$\Pr\{P_{obs}\downarrow \mid A\downarrow\}$	$\Pr\{A\downarrow \mid P_{obs}\downarrow\}$
5	0.000	0.000	0.200	0.333
10	0.600	0.750	0.639	0.793
15	0.697	0.852	0.789	0.849
20	0.786	0.911	0.838	0.928

*Two-tailed t test.
†One-tailed t tests.

adults, $\Pr\{A\downarrow\}$, or in observed parr, $\Pr\{P_{obs}\downarrow\}$, in the model substantiates this idea. Both probabilities increase as ρ increases. The effect is stronger, and increases more quickly, for longer censuses, w, when there is more opportunity to pick up a trend. In other words, there is an interaction between w and ρ. There is also an interaction with the observational uncertainty; as σ_{obs} increases, the true adult trend is unaffected (as is the true parr trend), yet we are less likely to observe it clearly. Hence, the probability of a true adult trend, $\Pr\{A\downarrow\}$, tends to be higher than the probability of an observed parr trend, $\Pr\{P_{obs}\downarrow\}$. The probability of both occurring, $\Pr\{A\downarrow, P_{obs}\downarrow\}$, thus closely mirrors that of the observed parr. For this reason, in general, $\Pr\{A\downarrow|P_{obs}\downarrow\} > \Pr\{P_{obs}\downarrow|A\downarrow\}$ for any particular parameter combination (e.g., see Table 2; Figs. 6 and 7).

Interaction of survey length, autocorrelation, and observational uncertainty

More detailed contour plots (Figs. 6 and 7) show that the factors w, ρ, and σ_{obs} interact to determine statistical power. Thus, for a given ρ, we can assess whether it is more useful to improve sampling methods or to increase census times in order to be able to observe population trends at a required level of statistical power. The best approach to answering such a question depends strongly on the question itself. For example, a comparison of Figs. 6 and 7 indicates that our power to observe a parr decline given a decline in true adult numbers increases relatively slowly as the census time w is increased. There is a strong improvement between $w = 5$ and $w = 10$ but much less between $w = 15$ and $w = 20$. On the contrary, increasing w substantially improves our ability to infer a decline in true adult numbers given an observed decline in parr.

The best way to improve power also depends on the values of these parameters and on our ability to improve them. For coho salmon, very little is known about the values of ρ (over which value we have no influence) and σ_{obs} (which value we may be able to improve in future census work); this was, in part, the motivation of this study. Preliminary estimates from coho data from the Oregon Production Area indicate that ρ is approximately 0.29. From marine survival data on the observation of adult numbers, σ_{obs} may be about 0.4 (Berman 1999). These values are not strictly applicable to the system in question (Californian coho populations

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Fig. 4. $\Pr\{P_{obs}\downarrow|A\downarrow\}$ and $\Pr\{A\downarrow|P_{obs}\downarrow\}$ versus σ_{obs} for the case where $\rho = 0.29$. (*a* and *e*) $w = 5$; (*b* and *f*) $w = 10$; (*c* and *g*) $w = 15$; (*d* and *h*) $w = 20$.

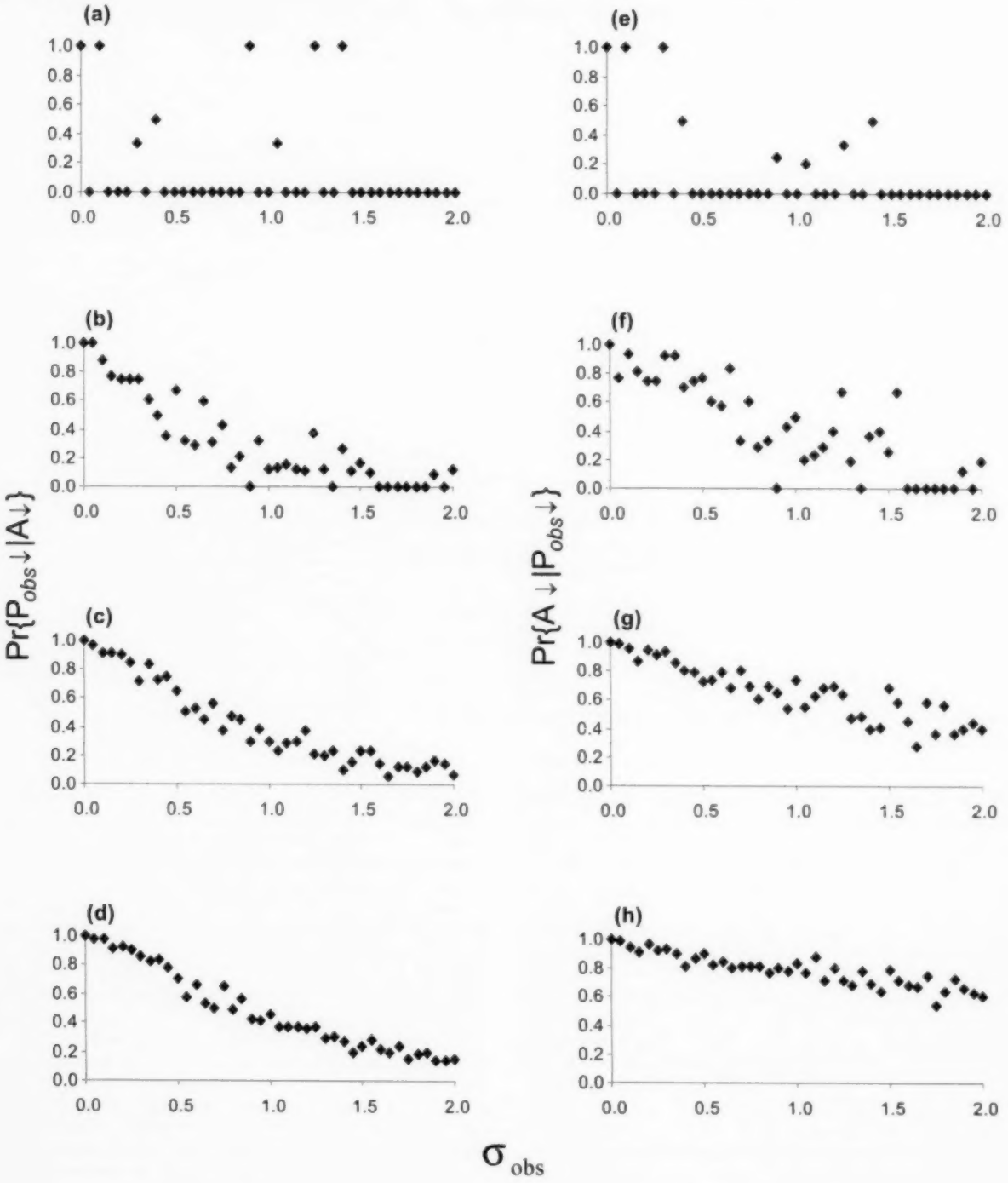

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Fig. 5. $\Pr\{P_{obs}\downarrow|A\downarrow\}$ and $\Pr\{A\downarrow|P_{obs}\downarrow\}$ versus ρ for the case where $\sigma_{obs} = 0.4$. (*a* and *e*) $w = 5$; (*b* and *f*) $w = 10$; (*c* and *g*) $w = 15$; (*d* and *h*) $w = 20$.

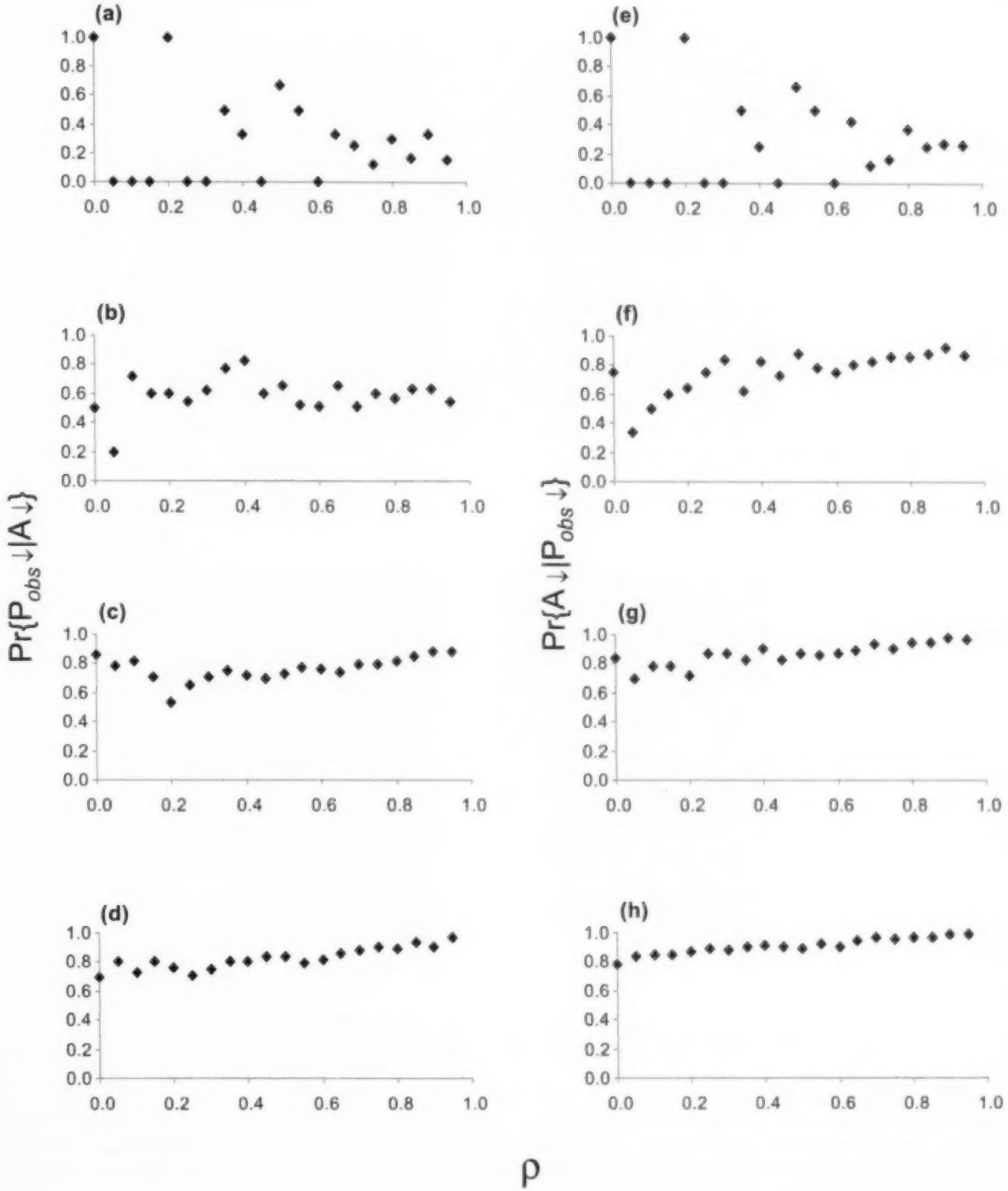

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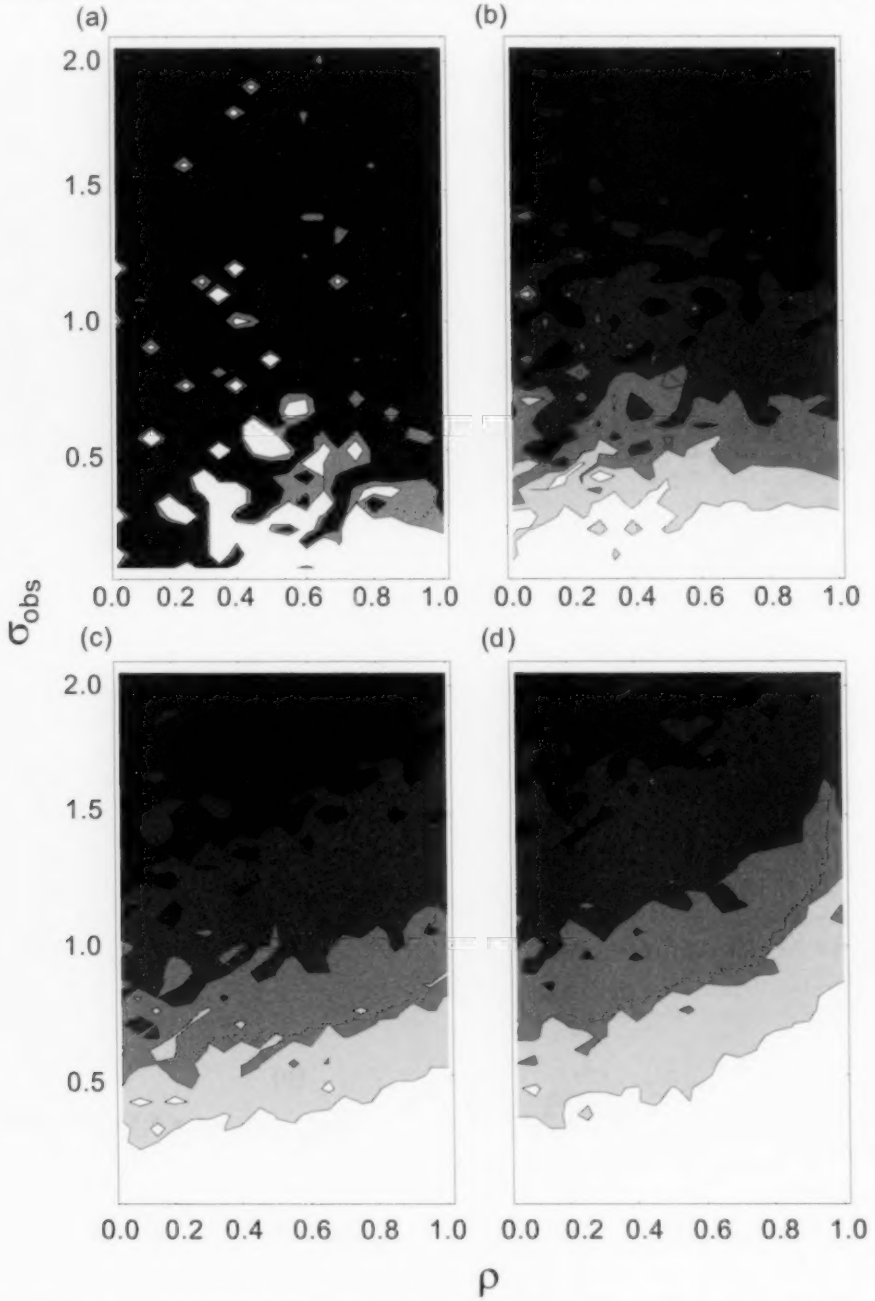
Fig. 6. Contour plots for $\Pr\{P_{obs}\downarrow|A\downarrow\}$ showing σ_{obs} against ρ for the case where (*a*) $w = 5$, (*b*) $w = 10$, (*c*) $w = 15$, and (*d*) $w = 20$. Contours are plotted at $\Pr\{P_{obs}\downarrow|A\downarrow\} = 0.2$, 0.4, 0.6, and 0.8 with shading from black at $\Pr\{P_{obs}\downarrow|A\downarrow\} < 0.2$ to white at $\Pr\{P_{obs}\downarrow|A\downarrow\} > 0.8$.



where observations are made on juvenile fish in freshwater); however, conditional probabilities based on these values provide an indication of the potential statistical power available (Table 2). Note that the results depend also on the placing of the burden of proof. For the case where we asked whether a change in population size was detected (using one two-tailed *t* test, i.e., 2.5% in each tail), the probabilities are in general lower than for the cases where we asked whether a positive or negative trend was detected (using two one-tailed *t* tests, 5.0% in each tail). These results are consistent across the entire parameter space presented here.

Discussion

Discerning population trends, especially for endangered aquatic species, is difficult. The work that we present here

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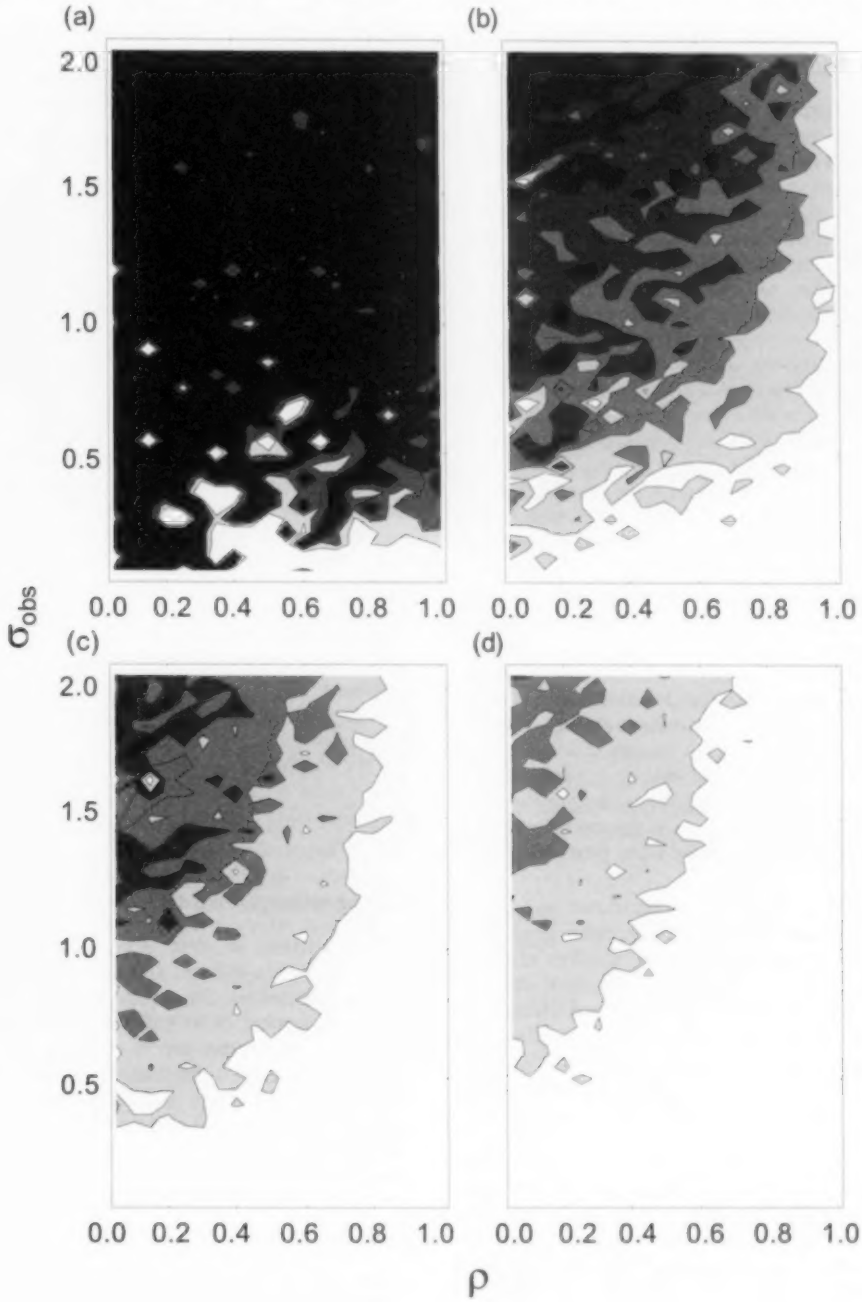
Fig. 7. Contour plots for $\Pr\{A \downarrow | P_{obs} \downarrow\}$ showing σ_{obs} against ρ for the case where (*a*) $w = 5$, (*b*) $w = 10$, (*c*) $w = 15$, and (*d*) $w = 20$. Contours are plotted at $\Pr\{A \downarrow | P_{obs} \downarrow\} = 0.2$, 0.4, 0.6, and 0.8 with shading from black at $\Pr\{A \downarrow | P_{obs} \downarrow\} < 0.2$ to white at $\Pr\{A \downarrow | P_{obs} \downarrow\} > 0.8$.



asks how best to achieve our observation goals for coho salmon in the face of biological and observational uncertainties.

Our results show that statistical power decreases as observational uncertainty increases, as population censuses decrease in length, and as autocorrelation in mortality rates decreases. The power to observe populations clearly also depends on the level of statistical significance used. We addressed this in our examination of results for different burdens of proof (also see Peterman and Bradford 1987; Peterman 1990) rather than in the context of standard time series analysis (Box and Jenkins 1976) because the burden of proof is a more relevant framework for conservation problems. Our two questions were "given an observed decline in parr

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numbers, what is the chance that this reflects a decline in adults?" and "given that adult numbers are declining, what is the chance that we see that decline?" We have presented measures of statistical power for these questions, spanning the entire reasonable state space for the parameters w, ρ, and σ_{obs}.

These results generate several clear recommendations for managers wishing to improve detection of population trends and hence their ability to manage coho salmon in California. The most obvious recommendation is, of course, to acquire longer time series of data. We have shown that, other factors aside, the chance of detecting trends in population abundance is, in general, increased the longer the time series of data available. However, our results also provide estimates of statistical power to detect trends following an environmental change such as a regime shift or some other large-scale disturbance. A regime shift is a climatically induced shift in productivity, and there is growing evidence that such changes occur approximately every 30 years (MacCall 1996; Beamish et al. 1999). Population trends before and after such a shift are unlikely to be closely related. For this reason, even long time series may need to be truncated if evidence of a regime shift occurs, with concomitant loss of power. This work shows how markedly our ability to discern trends will decrease following such a shift.

This loss of power can, of course, be ameliorated by minimizing observational uncertainty, σ_{obs}. Just as increasing observational window length increases power, so does decreasing observational uncertainty. A further recommendation also arises: it is vital to have an accurate estimate of the level of uncertainty itself. This conclusion also applies to ρ; knowing its value is important, even though this is not a parameter that can be directly manipulated by a manager. Only with accurate estimates of ρ and σ_{obs} for coho salmon in California will a manager be able to assess the statistical power available for the detection of trends from time series of a particular length.

Analogous results, relating the observational uncertainty in abundance estimates to the number of samples required to estimate the cohort replacement rate (the number of future spawners produced by each spawner), have been obtained for other Californian salmonids (Botsford and Brittnacher 1998). Their work complements ours in the sense that their conclusions pertain to the specifics of management (what is the probability of extinction of the stock, under given assumptions?), while our work deals with the operational considerations that managers face (what is the chance of correctly observing a trend, under given assumptions?). Both their study and ours show that for a required level of statistical certainty, and for known ρ, observational uncertainty and observation window length must be considered simultaneously in the design of any sampling regime. Thus, in order to obtain meaningful results, it is necessary to frame any question explicitly, including an indication of where the burden of proof will lie. As we demonstrate, different questions can be answered with different levels of statistical power when all other parameters are the same.

Our results are based on a simplified model for coho life history. This model is in fact a special case of the generic model presented in this study. The generic model comprises a conceptual suite of models that can incorporate many or all factors addressed in previous theoretical work on Pacific salmon (e.g., Emlen 1995; Nickelson and Lawson 1998; Spencer 1999). This suite of models can incorporate all the sources of variability that have been deemed important in populations of salmon (genetic, environmental, demographic). For example, demographic/genetic effects could be modeled with depensatory survivorship. Coupled versions of the model would allow different rivers, with different life history parameters, linked by straying individuals, to be modeled. Regime shifts could be explicitly modeled using a two-state Markov model. Catastrophes could be modeled by sudden increases in mortality rates across all stages. All parameters can be made functions of time and also functions of density measures at those times as required. A further extension would be to use the models to carry out population viability analyses in order to assess relative extinction risk, either without intervention or under different management regimes.

The fact that we have a suite of models, rather than just a single model, will allow an ongoing research agenda to investigate exactly which additional information would be most usefully acquired. In the specific version of the model presented in this paper, we have looked at the importance of observational uncertainty and autocorrelation in mortalities. Different simplifications of the general model could be used to ask questions about the importance of different structural components of coho life history. Our next immediate question concerns the effects of different types of density dependence (K. Shea and M. Mangel, unpublished data). Looking only at a single model begs the question of whether the results seen are somehow dependent on the biology that has been included and that which has been left out. By comparing related models, we can explicitly address that question, something that has not systematically been done before for coho.

Acknowledgements

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Appendix. Specific coho salmon model

State variables

$P(t)$ = density of parr at time t

$S(t)$ = density of smolts at time t

$A_1(t)$ = density of one-sea-winter adults at time t

Parameters

Fecundities

f_1 = number of eggs laid by one-sea-winter females

Survivorships (see mortality below)

$s_p(t)$ = survivorship of parr at time $t-1$ to smolts at time t

$s_{RO}(t)$ = survivorship of smolts moving from the river to the ocean at time t (emigration survivorship); this can be thought of as a product of river migration survivorship and early ocean survivorship

$s_O(t)$ = survivorship of salmon in the ocean over one winter from time $t-1$ to time t

$s_{OR}(t)$ = survivorship of salmon moving from the ocean to the river at time t (immigration survivorship)

Survivorships and mortality

The four annual survivorships, $s_i(t)$, are related to the instantaneous mortality rates, $M_i(t)$, by

$$s_i(t) = e^{-M_i(t)}.$$

Instantaneous mortality is correlated from year to year with autocorrelation parameter ρ using a mixing model of the form

$$M(t) = \rho M(t-1) + (1-\rho)Z(t)$$

$$M(0), Z(t) \sim N(\overline{M}, \sigma_M^2).$$

Thus, there is a contribution, ρ, by the previous year's mortality and a contribution, $(1-\rho)$, from the random variable $Z(t)$.

Equations

For parr:

$$P(t) = \frac{1}{2} f_1 A_1(t-1).$$

A 50:50 sex ratio is assumed. Eggs are produced only by females. Variation in fecundity is not included.

For smolts:

$$S(t) = P(t-1)s_p(t).$$

For one-sea-winter adults:

$$A_1(t) = S(t-1)s_{RO}(t-1)s_O(t)s_{OR}(t).$$

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Effects of turbidity on prey consumption and growth in brook trout and implications for bioenergetics modeling

John A. Sweka and Kyle J. Hartman

Abstract: Brook trout (*Salvelinus fontinalis*) were held in an artificial stream to observe the influence of turbidity on mean daily consumption and specific growth rates. Treatment turbidity levels ranged from clear (<3.0 nephelometric turbidity units (NTU)) to very turbid water (>40 NTU). Observed mean daily specific consumption rates were standardized to the mean weight of all brook trout tested. Turbidity had no significant effect on mean daily consumption, but specific growth rates decreased significantly as turbidity increased. Brook trout in turbid water became more active and switched foraging strategies from drift feeding to active searching. This switch was energetically costly and resulted in lower specific growth rates in turbid water as compared with clear water. Bioenergetics simulations were run to compare observed growth with that predicted by the model. Observed growth values fell below those predicted by the model and the difference increased as turbidity increased. Abiotic factors, such as turbidity, which bring about changes in the activity rates of fish, can have implications for the accuracy of predicted growth by bioenergetics models.

Résumé : Des Ombles de fontaine (*Salvelinus fontinalis*) en ruisseau artificiel ont servi à l'étude de l'effet de la turbidité sur la consommation moyenne quotidienne et les taux spécifiques de croissance. Les conditions d'élevage variaient d'une eau claire (< 3.0 unités néphélométriques, NTU) à une eau très turbide (> 40 NTU). Les taux spécifiques quotidiens moyens de consommation observés ont été corrigés pour tenir compte de la masse moyenne de tous les ombles étudiés. La turbidité reste sans effet significatif sur la consommation quotidienne moyenne; en revanche, les taux spécifiques de croissance déclinent lorsque la turbidité augmente. Les ombles s'activent plus dans les eaux turbides et changent leur stratégie de recherche de nourriture, délaissant l'alimentation dans la dérive pour une recherche active de proies. Ce changement s'avère coûteux en énergie et entraîne un taux spécifique de croissance moins élevé en eau turbide. Des simulations bioénergétiques ont permis de comparer la croissance observée avec celle prédite par le modèle. Les taux observés étaient inférieurs aux taux prédits par le modèle et la différence entre les taux croissait avec l'augmentation de la turbidité. Les facteurs abiotiques, tels la turbidité, qui modifient les taux d'activité des poissons, peuvent ainsi affecter la précision des prédictions des taux de croissance par les modèles bioénergétiques.

[Traduit par la Rédaction]

Introduction

Salmonids are visual predators and turbidity is believed to play a significant role in their foraging efficiency. Steelhead (*Oncorhynchus mykiss*) and coho salmon (*Oncorhynchus kisutch*) have shown decreased growth rates when reared in turbid water (Sigler et al. 1984). Also, coho salmon actively avoid turbid waters (Bisson and Bilby 1982). Reactive distance of rainbow trout (*Oncorhynchus mykiss*) has been found to decrease linearly (Barret et al. 1992) and the reactive distance of brook trout (*Salvelinus fontinalis*) has been found to decrease exponentially as turbidity increases in artificial stream channels (Sweka 1999).

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Although decreased growth rates in turbid waters have been attributed to an inability to detect prey and subsequent decrease in consumption, few studies have quantified daily consumption in salmonids under turbid conditions. Brook trout have been shown to become more active in turbid waters (Gradall and Swenson 1982), which could be a mechanism promoting increased feeding activity during periods of high invertebrate drift in natural streams. However, in another drift feeder, Arctic grayling (*Thymallus arcticus*), a decrease in stomach contents was found in fish in turbid waters despite increased invertebrate drift (Birtwell et al. 1984).

Activity represents a significant portion of a fish's energy budget. Changes in activity confound predictions of bioenergetics models because the activity component of the model is assumed to be constant (Boisclair and Leggett 1989). Kerr (1982) hypothesized that the energy allocated to activity was positively related to the quantity of food consumed for actively foraging fish. This was also confirmed by Boisclair (1992), who found a positive logarithmic relationship between activity rates and feeding rates. These findings were based on observations of fish in still water. Brook trout typically utilize a drift-feeding strategy in streams, whereby they hold in a single position or focal point, leave the focal point to intercept prey, and then return to the original focal

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point (Fausch 1984; Grant 1990). Drift-feeding fish should select focal points in areas of minimal water velocity, yet close to areas of swift current, thus minimizing the energy required to maintain position and maximizing access to invertebrate drift (Fausch 1984). Such areas would be those close to obstructions or close to the streambed where current velocity is lowest (Allan 1995). Under a drift-feeding strategy, the current velocity at the focal point governs activity and the energy required in maintaining position. However, Gradall and Swenson (1982) found that brook trout become more active under turbid conditions and were found less in association with the substrate. This would indicate that brook trout abandon a theoretical optimal foraging strategy when exposed to turbid waters. This increase in activity could increase activity costs not only due to increased movement but also due to exposure to higher current velocities typically found away from the substrate. Abiotic factors, such as turbidity, that alter the activity of fish could have profound effects on the accuracy of predictions from bioenergetics models.

Brook trout are the top predators in Appalachian headwater stream communities and are often the only fish species present in such streams. Land-use practices such as timber harvest, mining, and road construction may result in increased sediment loads in headwater streams. Although much research has focussed on the effects of deposited sediment as a limiting factor for brook trout spawning potential, turbidity may be an important yet relatively unexamined factor also contributing to the perceived decline of brook trout production in Appalachian streams. In this study, we sought to test three hypotheses regarding potential mechanisms whereby turbidity may affect brook trout growth: through (*i*) reduced consumption, (*ii*) increased activity, (*iii*) or a combination of both. Finally, we discuss the implications of our results for bioenergetics modeling of stream-dwelling salmonids.

Materials and methods

All experiments were conducted in an artificial stream. Brook trout were tested at sequential treatment levels of turbidity ranging from clear water (<3.0 nephelometric turbidity units (NTU)) to >40 NTU. Each trial lasted at least 5 days with five to six trials at different turbidity levels conducted on each fish. A subsample of the brook trout tested was videotaped while foraging to quantify changes in activity with increasing turbidity.

Artificial stream design

Consumption and growth experiments were conducted in a 1.2 × 2.4 m steel tank (Fig. 1). A 1.8-m removable baffle was installed, separating the tank into two 0.6-m-wide sections with fences (1-cm^2 mesh) at each end. One fish was placed in each of the sections. The artificial stream was surrounded by a curtain to minimize outside disturbance. Current (0.06 $m \cdot s^{-1}$) was created with three powerhead pumps placed in front of each section. Drift nets were placed downstream of the fencing to catch uneaten prey. The light intensity within the artificial stream was 49 lx with a 14 h light : 10 h dark photoperiod. Water temperatures were maintained at 12.0 ± 0.5°C (mean ± SE) within the artificial stream. Water was chilled in a head tank with a 16-A chiller, and a 6-A submersible pump was used to deliver chilled water to the artificial stream.

Fish

Brook trout were obtained from Bowden State Fish Hatchery, Bowden, W.V., U.S.A. Overall mean total lengths at the beginning of testing in clear water were 126 mm (range 77–153 mm) and mean weights were 22.23 g (range 4.63–35.38 g) (Table 1). A wide range of fish sizes was used to determine the effect of size on consumption and to estimate allometric consumption parameters. These allometric consumption parameters were later used for standardization of specific consumption rates to the overall mean body weight of brook trout across trials. Brook trout energy density was estimated from percent dry weight mass of a separate sample of 15 fish using lake trout (*Salvelinus namaycush*) energy constant regression parameters as in Hartman and Brandt (1995*a*). Mean energy density was 6481.3 ± 156.3 J·g wet $weight^{-1}$ (mean ± SE).

Prey

Trials were conducted using live housefly (*Musca domestica*) larvae as prey. Larvae were 10.3 ± 0.2 mm in length and had an energy density of 8426.4 ± 90.4 J·g wet $weight^{-1}$ as calculated from Cummins and Wuycheck (1971). Air was injected into the larvae with a syringe before being offered to the brook trout, making the larvae float and insuring that any unconsumed prey entered the drift net for retrieval. To be sure that the larvae would float, they were dropped in a container of water and only those that floated were offered to the brook trout.

Experimental procedure

Unlike other growth experiments in turbid water, which measured growth in groups of salmonids (i.e., Sigler et al. 1984), we observed daily consumption and growth in individual brook trout. We felt that mean consumption, determined by measuring consumption in groups of fish, would be biased due to possible competitive dominance by a few individuals. Before beginning experimentation, each brook trout was anesthetized with tricane methanesulfonate (MS 222), weighed on an analytical balance to the nearest 0.01 g, total length measured to the nearest millimetre, and placed into one of the sections of the artificial stream. Individuals were allowed to acclimate to the testing environment for 12 h before testing began.

Turbidity treatment levels were created by mixing sediment collected from the banks of a local stream in a bucket and adding the solution to the head tank until a desired treatment level was reached. Fish were initially held at given treatment for a duration of 7 days and fed four times daily. A pilot study in clear water showed that trials could be shortened to a duration of 5 days and two feedings per day with no difference in mean daily consumption. This simplified experimental logistics and shortened experimental time. During each feeding period, three turbidity measurements were taken with a Lamotte 2020 turbidimeter and the median recorded. Also, during nontesting periods throughout the day, turbidity was periodically measured. When turbidity began to fall below a desired level, the head tank was agitated to resuspend sediment. At any time, turbidity did not deviate from a desired level by more than 4 NTU. Two standards, 5 and 10 NTU, and distilled water were used to calibrate the turbidimeter between treatments.

Prey items were offered to the brook trout in an amount greater than the maximum consumption predicted for lake trout of similar sizes at experimental temperatures. Consumption parameters from Stewart et al. (1983) were used in Fish Bioenergetics 3.0[8] (Hansen et al. 1997) to determine maximum consumption, and brook trout were offered an amount greater than the value predicted by the model each day. Maximum consumption values predicted by the model were divided in half to arrive at the appropriate weight to offer the fish in each of the two feeding periods per day. For small fish (<10 g), predicted mean daily maximum consumption (C_{max}) =

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Fig. 1. Overhead view of artificial stream design. See Materials and methods (Artificial stream design) for a description.

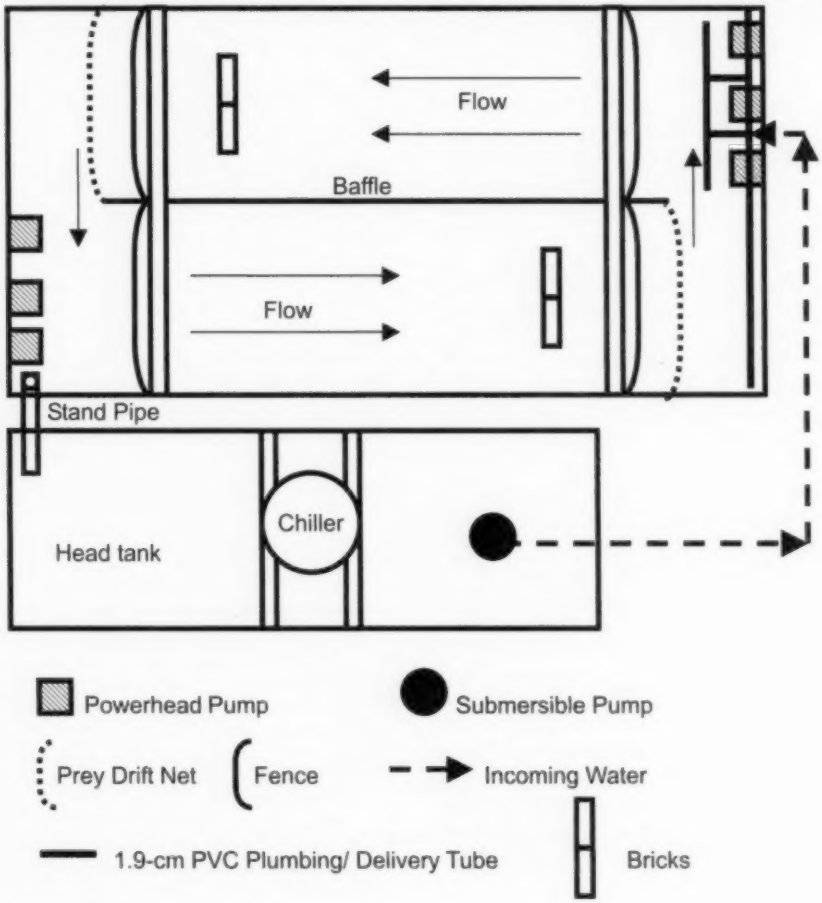

Table 1. Experimental treatments.

Turbidty (NTU)	*N*	Start weight (g)	End weight (g)	Consumption ($g \cdot g^{-1} \cdot day^{-1}$)	Growth ($g \cdot g^{-1} \cdot day^{-1}$)
0.85 (0.06)	10	22.23 (4.63–35.38)	28.48 (6.16–45.54)	0.07 (0.01)	0.05 (0.01)
6.14 (0.64)	3	9.12 (6.16–13.20)	11.34 (7.68–16.71)	0.08 (0.02)	0.03 (0.01)
10.39 (0.21)	7	24.91 (7.68–39.99)	32.17 (10.38–50.02)	0.09 (0.01)	0.05 (0.01)
15.18 (0.31)	6	28.74 (11.76–45.54)	33.78 (13.28–52.72)	0.06 (0.01)	0.03 (0.01)
20.02 (0.25)	7	35.52 (13.27–50.02)	41.48 (15.54–59.03)	0.07 (0.01)	0.03 (0.01)
25.27 (0.43)	6	32.84 (15.12–51.83)	41.19 (17.66–66.74)	0.06 (0.01)	0.05 (0.02)
29.87 (0.30)	4	53.64 (45.19–59.03)	60.38 (49.60–65.75)	0.06 (0.01)	0.03 (0.01)
35.38 (0.72)	3	51.74 (35.84–66.74)	56.74 (38.07–71.22)	0.05 (0.02)	0.02 (0.01)
39.98 (0.51)	4	60.38 (49.60–65.75)	65.88 (57.04–70.83)	0.07 (0.01)	0.02 (0.01)
44.75 (0.87)	3	56.64 (37.77–71.22)	61.19 (41.12–75.62)	0.04 (0.02)	0.02 (0.01)

Note: Turbidity is represented as the mean ± 95% CI during an experimental trial. *N* is the number of fish tested at the given turbidity level. Ten individual fish were tested and each was tested at sequentially increasing levels of turbidity. Start weights and end weights indicate mean size and the size range of fish tested at a given level of turbidity. Consumption and growth values are means ± 95% CI at a given level of turbidity. Note that consumption values are observed means. In the statistical analysis, individual means were adjusted to the overall mean weight of all fish tested to account for allometric changes in consumption.

0.13 $g \cdot g^{-1} \cdot day^{-1}$ (range 0.12–0.15 $g \cdot g^{-1} \cdot day^{-1}$). Therefore, during a single feeding period, the weight of prey offered was greater than the predicted C_{max} times the starting weight of the fish divided by 2. The amount of prey offered to small fish averaged 2.11 $g \cdot day^{-1}$ (1.05 g·feeding $period^{-1}$) with approximately 25 $larvae \cdot day^{-1}$ (12–13 $larvae \cdot feeding\ period^{-1}$). The mean weight (±SE) of an individual larvae was 0.076 ± 0.001 g. As the fish grew, more prey was offered to them. For large fish (<40 g), predicted mean daily C_{max} = 0.07 $g \cdot g^{-1} \cdot day^{-1}$ (range 0.06–0.07 $g \cdot g^{-1} \cdot day^{-1}$). The amount of prey offered to large fish averaged 5.22 $g \cdot day^{-1}$ (2.6 g·feeding $period^{-1}$) with approximately 84 $larvae \cdot day^{-1}$ (42 $larvae \cdot feeding\ period^{-1}$). Larvae were offered to the fish by rinsing them down a delivery tube at the front of each section of the artificial stream. Once a prey item was offered, another was not offered until the

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previous one was consumed or drifted though the back of the section into the drift net. Larvae were offered at a rate of one every 30–45 s and with the current velocity of 0.06 $m \cdot s^{-1}$, it took approximately 20 s for prey to drift through the tank if they were not consumed

Daily consumption was estimated as the difference between the total weight of prey offered and that recovered. After all prey were offered, uneaten prey were collected from the drift nets, blotted dry, and weighed once more to calculate the amount consumed by each fish. Brook trout never consumed all the prey that was offered. Feeding took place between 07:00 and 08:00 for morning feedings and between 19:00 and 20:00 for evening feedings. Each feeding period lasted for approximately 25–45 min, depending on the amount of prey that had to be offered.

To standardize the rate of gastric evacuation, fish were not fed for 12 h before initial weighing and were given 12 h following the final feeding period in a given treatment before reweighing to determine growth over the trial duration. With the 12-h fasting period, we assumed the same degree of gastric evacuation prior to and following a treatment. Differences between initial and final weights would thus be due to growth and not due to undigested food at the end of a treatment duration. Average specific growth rates (grams per gram per day) over the treatment duration were calculated as the difference between start and end weights divided by the start weight and divided the number of days. Each fish was then placed back into its respective section of the artificial stream and tested at the next highest treatment turbidity level. The weights and lengths at the end of the previous trial were used as the starting weights and lengths at the beginning of the next trial (i.e., the end weight of trial n equaled the start weight of trial $n + 1$).

A total of 10 fish were tested. While testing the first three fish, treatment turbidity levels ranged from clear water to approximately 25 NTU with mean treatment levels of turbidity at 0.8, 6.1, 10.1, 14.8, 20.3, and 25.6 NTU. We initially felt that this would be high enough to show a difference in mean daily consumption, as Sigler et al. (1984) found that this level was enough to cause a reduction in coho salmon growth. However, we did not observe a significant change up to 25 NTU (see Results), so we then tested fish at turbidity levels ranging from clear water to >40 NTU (Table 1) with five treatment levels run on each fish to see if turbidity would have an effect at these higher levels.

Movement experiment

Visual observation of the brook trout feeding showed that as treatment turbidity increased, the fish began to move more often and to actively search for prey. To quantify movement, the last four fish tested were videotaped during feeding experiments. Each fish was videotaped during three randomly chosen feeding periods throughout each treatment using a Sharp E550 View Cam mounted above each section. Turbidity levels for each trial were approximately 0.6 (clear water), 10, 20, 30, and 40 NTU. In review of the videotape, frame capture software (Multimedia Studio Pro®) was used to capture a particular frame at the instant a fish first recognized a prey item. A computer-generated grid (32 quadrants, each 18 × 18 cm) was then transposed onto the section of the tank and the location of the fish when recognizing a prey item was recorded. We assumed that the typical feeding strategy was "drift feeding" where a fish would hold in a single location, attack a prey item, and then return to the original holding position. For an index of activity, we enumerated the number of quadrants used to attack prey from. An increase in the number of quadrants used to attack prey would indicate abandonment of the drift-feeding strategy. Videotaped feeding trials were approximately 30 min in duration.

Statistical analysis

Nonlinear regression was used to determine the effect of body size on mean daily consumption for brook trout in clear water (the control). The allometric consumption model was used to describe the relationship between consumption and body size (Kitchell et al. 1977):

$$C = CA(W^{CB})$$

where C is consumption (grams per gram per day), CA is the intercept of the allometric mass function, and CB is the slope of the allometric mass function. Because individual fish were tested over several weeks, and grew as testing continued, the CA and CB parameters found in clear water were used to standardize observed daily consumption values to the overall mean weight of all fish used in all experiments as in Hartman and Brandt (1995*b*).

Although two fish were tested at once (each in one half of the artificial stream) and shared the same water, we treated each fish as an independent observation. Both halves of the artificial stream were identical, both fish were fed at the same time, and neither fish had any contact with the one in the other half of the artificial stream. Therefore, we have no reason to believe that one fish may have influenced the foraging behavior of the other. Because each fish was tested at a sequence of treatments, we analyzed the effect of increasing turbidity on mean specific consumption and average specific growth rates as a randomized block analysis of variance (ANOVA) with individual fish as blocks. Indices of activity (the number of quadrants used to forage) were also analyzed as a randomized block ANOVA. Significance was set at an alpha level of 0.05.

Simulations were run using Fish Bioenergetics 3.0® software to compare observed growth rates with those predicted by the model for lake trout. All lake trout respiration, digestion, and excretion parameters were used in the simulations (Stewart et al. 1983). We used lake trout parameters because of the close systematic relationship between brook trout and lake trout (both are in the same genus) and the similar temperature requirements for each species. The CA and CB consumption parameters determined for brook trout in clear water (this study) replaced those of the lake trout. We assumed that 10% of the prey wet weight was indigestible. The model was fit to observed total consumption from the laboratory experiments, and differences between observed and predicted values for growth at the end of a trial were analyzed using a randomized block ANOVA with individual fish as blocks.

Results

Experiments in clear water showed a weak relationship between mean daily consumption and weight of the brook trout. Larger fish had lower specific consumption rates than smaller fish. The CA and CB parameters for the allometric equation $C = CA(W^{CB})$ were 0.130 and –0.201, respectively (Fig. 2).

The experimental allometric consumption parameters were then used to standardize brook trout consumption to the overall mean weight (±SE) of fish tested (37.44 ± 6.02 g). Adjusted mean daily consumption differed significantly between individual brook trout ($F = 5.10$, $p < 0.01$) (Fig. 3), but there was no effect of increasing turbidity ($F = 0.49$, $p = 0.48$). Because of the relatively poor association between mean daily consumption in clear water and mean fish weight ($r^2 = 0.12$) and the large difference between lower and upper 95% confidence intervals (CI) for the CB parameter (lower 95% CI = –0.344, upper 95% CI = –0.058), we also standardized mean daily consumption rates using lake trout CA and CB parameters (CA = 0.0589 and CB = –0.307; Stewart et al. 1983). Again, turbidity showed no significant influence on mean daily consumption ($F = 0.236$, $p = 0.63$).

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Fig. 2. Regression of mean daily consumption (C) on mean weight (W) of brook trout tested in clear water. The 95% CI on CA was ±0.052 and the 95% CI on CB was ±0.143.

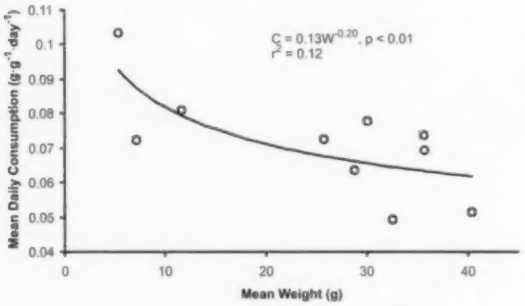
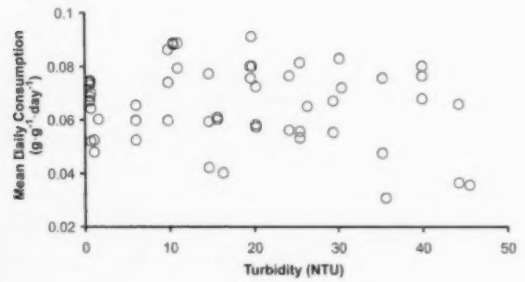


Fig. 3. Regression of standardized mean daily consumption values on mean turbidity level for each trial. Although individual fish differed from each other ($F = 5.10$, $p < 0.01$), turbidity did not have a significant influence on mean daily consumption ($F = 0.49$, $p = 0.48$).

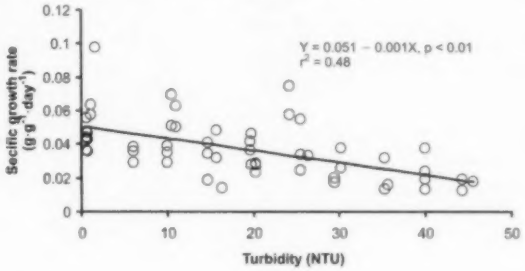


Specific growth rates decreased significantly with increasing turbidity ($F = 33.87$, $p < 0.01$) (Fig. 4) and individual fish were not significantly different from each other ($F = 0.61$, $p = 0.78$). Mean specific growth rates in clear water were 0.053 ± 0.013 $g \cdot g^{-1} \cdot day^{-1}$ (mean ± 95% CI), while those at the highest treatment levels (≥38 NTU) were 0.020 ± 0.006 $g \cdot g^{-1} \cdot day^{-1}$. Brook trout specific growth rates thus showed a 62% decrease from clear water to the highest treatment levels.

The movement study showed that the number of quadrants within each section of the artificial stream used to forage increased significantly with increasing turbidity ($F = 28.83$, $p < 0.01$) (Fig. 5), despite much variation between fish at intermediate treatment levels (10–20 NTU). Individual fish also differed between one another ($F = 14.83$, $p < 0.01$). In clear water, 75% of the fish used three or fewer quadrants to forage, while one of the fish tended to move more frequently and forage from various locations in clear as well as turbid water.

Bioenergetics simulations gave predicted trial end weights for each fish based on the total amount of prey consumed. Observed values consistently fell below those predicted by the model and the difference increased as turbidity increased ($F = 32.22$, $p < 0.01$) (Fig. 6) and individual fish differed from one another ($F = 4.66$, $p < 0.01$). Mean observed growth in clear water was 35% lower than that predicted by the bioenergetics model, while mean observed growth in the highest treatment level (NTU > 40) was 55% lower than that predicted by the bioenergetics model.

Fig. 4. Regression of specific growth rate (Y) on mean trial turbidity (X). Specific growth rates decreased significantly with increasing turbidity ($F = 33.87$, $p < 0.01$). Individual fish did not differ from each other ($F = 0.61$, $p < 0.78$).



Discussion

The results that we obtained were contrary to what would be expected from predictions of encounter rate models (Gerritsen and Strickler 1977). Turbidity had no significant influence on brook trout mean daily consumption. Reactive distance has a strong influence on encounter rates between predator and prey, and turbidity has been shown to decrease the reactive distance of fish (Barret et al. 1992; Miner and Stein 1996; Sweka 1999). According to the Gerritsen and Strickler (1977) model, decreased reactive distance leads to decreased encounter rates. For drift-feeding fishes like brook trout, decreased reactive distance under turbid conditions would be expected to decrease the volume of water searched per unit time. This should eventually result in decreased consumption rates.

One means by which a predator could compensate for a decrease in reactive distance would be to increase swimming activity. Gradall and Swenson (1982) found that in water of 7.1 formazin turbidity units (FTU), brook trout activity increased and association with cover and substrate decreased. (Benfield and Minello (1996) gave a conversion of FTU to NTU where FTU = 1.25·NTU. Therefore, 7.1 FTU = 8.8 NTU.) Increasing the time spent moving increases the chance of encountering potential prey by increasing the total volume of water searched. For brook trout in this study, a switch in foraging strategy was observed. In clear water, typical drift-feeding behavior (Grant 1990) was observed where a fish would recognize a prey item, strike at that prey item, and return to the original holding position. As the water became more turbid, fish tended to utilize more of the available area of the tank to forage. The number of locations used to forage increased significantly as turbidity increased. By using more locations as holding positions, the brook trout increased their chance of recognizing potential prey, thus compensating for decreased reactive distance with increasing turbidity. Although foraging locations were quantified for only four brook trout, we feel that their behavior is representative of all tested fish based on observation while conducting feeding trials.

The transition from a typical drift-feeding strategy to an active searching strategy took place between 10 and 20 NTU

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Fig. 5. Regression of the number of quadrants used to forage (Y) on turbidity (X). Each point represents the number of quadrants used by brook trout to forage during one videotaping session. The number of quadrants used to forage within the artificial stream increased significantly as turbidity increased ($F = 28.41$, $p < 0.01$). Individual fish also differed from one another in the number of quadrants used ($F = 14.83$, $p < 0.01$).

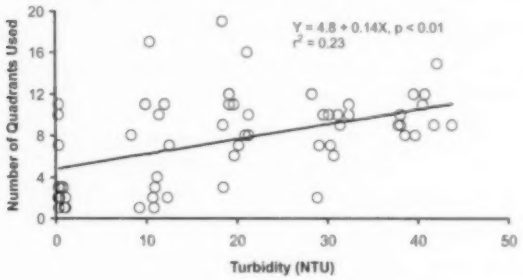
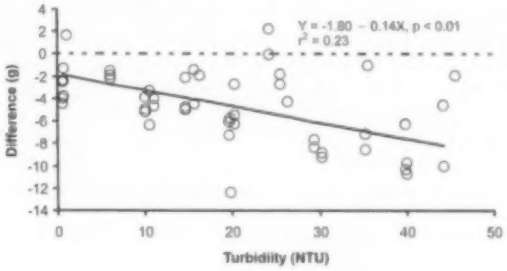


Fig. 6. Regression of the difference (Y) in observed trial end weights and those predicted from the bioenergetics model on turbidity (X). As turbidity increased, the difference between observed growth and that predicted by the model increased significantly, with observed values being lower than those predicted by the model ($F = 32.22$, $p < 0.01$).



where the greatest variation in the number of quadrants used to forage occurred. At these levels of turbidity, the fish were faced with a choice of holding in a single location, which may decrease the number of prey recognized, or utilizing more of the tank and increasing the chance of prey detection. At higher turbidity levels (30–40 NTU), lower variation in the data and a higher mean number of quadrants used suggest that active searching for prey was the primary foraging strategy.

Another possible mechanism for the observed increased activity may be a decrease in the perceived risk of predation by the fish. Gregory (1993) found juvenile chinook salmon (*Oncorhynchus tshawytscha*) to show less predator avoidance behavior as turbidity increased. Also, Gregory and Northcote (1993) found feeding rates of this species to increase at slightly elevated turbidity levels and they attributed this to a decrease in the potential risk to predators.

Although active searching for prey may compensate for decreased visual acuity in turbid waters, this strategy is energetically taxing. Net return per unit effort in foraging would be expected to decrease when switching from a typical drift-feeding strategy to an actively searching strategy as the activity required to capture each prey item increases (Boisclair and Leggett 1989; Boisclair and Sirois 1993). This can be seen in the decrease in brook trout specific growth rates with increasing turbidity. Reduced growth rates seen in fishes under turbid conditions may not only be a result of decreased visual acuity but also a result of the increased amount of energy required to locate prey.

The brook trout grew over treatment levels, and it may be argued that this could account for the decrease in specific growth rate. However, regression of specific growth rate on body weight for brook trout tested in clear water showed no correlation between fish size and specific growth rate ($F = 1.36$, $p = 0.28$). Therefore, we believe that the reduction in specific growth rates seen under turbid conditions is due to the treatment effect rather than possible allometric changes in growth rates.

Our results are limited to a single floating prey type, which may bring into question this study's applicability to the field. However, during some times of the year, as much as 50% of salmonid diets can come from terrestrial invertebrates (Wipfli 1997), which typically float on the surface of the water. Composition of aquatic invertebrates in the drift can also change as turbidity increases, with larger macroinvertebrates increasing their propensity to drift as light levels are reduced (Douglas et al. 1994; Waters 1995). The housefly larvae used in these experiments could be considered relatively large invertebrate prey. Brook trout have also been shown to be size selective, with overrepresentation of larger prey in their diets compared with the drift (Allan 1980). Given this selectively, and the propensity for larger invertebrates to drift under lower light levels, our results show how turbidity influences foraging abilities with respect to larger, more energetically profitable prey.

The bioenergetics model simulations illustrated the influence of turbidity on the energetic return per unit effort. In clear water, trial end weights were slightly below those predicted by the bioenergetics model. The 0.06 $m \cdot s^{-1}$ current velocity in the artificial stream not taken into account by the bioenergetics model may explain the difference. As turbidity increased, the difference between trial end weights predicted by the model and those observed increased significantly, thus indicating a more energetically costly situation as the brook trout switched from a drift-feeding strategy to an active searching strategy.

The comparison of observed growth with that predicted by the bioenergetics model points out simplification in the assumptions of the bioenergetics model. The model assumes constant rates of activity, which is seldom the case. Activity plays a significant role in a fish's energy budget (Boisclair and Sirois 1993). Changes in abiotic factors, such as turbidity, can influence fish behavior, leading to changes in activity independent of temperature fluctuations. These changes in activity can strongly influence the accuracy of predicted growth by bioenergetics models. With the difference between observed and predicted growth over the relatively short duration of the experiments in this study, caution should be exercised when using bioenergetics models to predict growth in natural populations of stream-dwelling fish over longer time periods, especially where turbidity is elevated or variable or where other abiotic conditions are common.

The use of lake trout metabolism parameters (Stewart et al. 1983) may have biased our simulation results, but this bias would be expected to be constant if turbidity had no

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effect on activity. In clear water, the predicted growth from the model was greater than that observed. This could be due to physiological differences between the species and differences in experimental design between our study and that of Stewart et al. (1983) in obtaining consumption parameters. Our purpose here was not to test the bioenergetics model or to use it to predict growth. We used the bioenergetics simulations as a tool to illustrate how turbidity can affect model predictions. If a complete brook trout model were available at the time of this study, the results would likely be similar due to changes in activity costs not accounted for in the model. As turbidity increased, the model predictions became less accurate, which we believe is a function of increased activity and not due to species differences in true bioenergetics parameters.

Decreased growth rates in turbid water in other salmonids have been attributed to decreased visual acuity and thus decreased consumption (Sigler et al. 1984). In another set of experiments, we found that the reactive distance of brook trout decreased exponentially with increasing turbidity (Sweka 1999). The decrease in reactive distance was expected to translate into a decrease in mean daily consumption. Although visual acuity may result in decreased consumption rates in salmonids, growth rates may not only be governed by consumption but also by activity. In this study, turbidity did not directly influence brook trout growth rates through reduced mean daily consumption but caused behavioral changes in activity, which were energetically costly.

Brook trout in the Appalachians inhabit relatively high-gradient streams and the amount of sediment deposition for a given load level is inversely related to gradient. Thus, increased sediment loading to streams may not be sufficient to alter habitat and substrate. However, increased sediment loading may markedly change mean stream turbidity levels (Barret et al. 1992). Thus, although spawning habitat and invertebrate biomass may be unaffected, turbidity can decrease growth rates in fish through decreased visual acuity and subsequent changes in feeding behavior in an effort to compensate for the decreased ability to detect prey under turbid conditions. Factors such as turbidity, and its influence on activity, complicate application of bioenergetics models describing stream-dwelling fish in the field, and an overestimation of potential growth may result. The results found here suggest that turbidity can decrease brook trout growth through allocation of energy for changes in activity rather than for growth.

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Why are dwarf fish so small? An energetic analysis of polymorphism in lake whitefish (*Coregonus clupeaformis*)

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Abstract: Sympatric populations of dwarf lake whitefish (*Coregonus clupeaformis*) (DLW) and normal lake whitefish (NLW) commonly occur in north temperate and subarctic lakes. DLW have a much lower growth, mature earlier, and have a shorter life span than NLW. Furthermore, they are usually not found when cisco (*Coregonus artedi*) are present, possibly due to competitive exclusion. In this study, we compared the energy budget of DLW, NLW, and cisco using food consumption rates estimated with mass balance models of chemical tracers (i.e., mercury and radiocesium). These chemicals are globally distributed and can be readily detected in fish and their prey. Our analysis showed that the energy budget of DLW and cisco was similar. DLW and cisco consumed on average 40–50% more food than NLW. The conversion efficiency of DLW and cisco was two to three times lower than that of NLW. These results suggest that DLW and cisco allocated a larger fraction of their energy budget to metabolism than NLW. Our analysis also suggests that the earlier maturation and shorter life span of DLW and cisco may be due to their higher metabolic rates.

Résumé : On retrouve fréquemment des populations sympatriques de corégones (*Coregonus clupeaformis*) nains (CNA) et normaux (CNO) dans les lacs tempérés et subarctiques. Les CNA ont une croissance plus faible, maturent plus rapidement et vivent moins longtemps que les CNO. De plus, on ne les retrouve habituellement pas en présence de ciscos (*Coregonus artedi*), possiblement due à la compétition. Dans cette étude, nous avons comparé le budget énergétique des CNA, des CNO et des ciscos à l'aide de taux de consommation estimés avec un bilan massique de traceurs chimiques (i.e., mercure et cesium radioactif). Ces éléments sont globalement distribués et peuvent être facilement détectés dans les poissons et leurs proies. Nos analyses indiquent que le budget énergétique des CNA et des ciscos était similaire. Les CNA et les ciscos consommaient en moyenne 40–50% plus de nourriture que les CNO. L'efficacité de croissance des CNA et des ciscos était deux à trois fois plus faible que celle des CNO. Ces résultats suggèrent que les CNA et les ciscos allouaient une plus grande proportion de leur budget énergétique au métabolisme que les CNO. Nos analyses suggèrent également que la maturation plus rapide et la courte longévité des CNA et des ciscos peuvent être arribuées à leurs demandes métaboliques plus élevées.

Introduction

Growth is a thermodynamic process that results from the balance between the energy consumed and the energetic costs associated with metabolism and fecal and waste products. When energy intake exceeds energetic costs, the surplus energy can be channeled to growth and reproduction. Once sexual maturity is reached, growth rate usually decreases in fish, as they allocate a fraction of their surplus energy to produce gonads and to fuel the behavioral activities associated with reproduction such as migration, courtship, and building and guarding nests (Roff 1983).

Growth is highly flexible in fish and varies tremendously both within and among populations (Boisclair and Leggett 1989). Sympatric populations of dwarf and normal ecotypes of the same species have been frequently observed in north temperate and subarctic lakes (Robinson and Wilson 1994; Pigeon et al. 1997). Dwarf fish are characterized by a much lower growth rate than normal fish. They reach maturity earlier and at a smaller size and also tend to have a much shorter life span than normally growing fish (Fortin and Gendron 1990; Bodaly et al. 1991). In addition, dwarf and normal phenotypes usually forage on different prey items and in different habitats (pelagic versus benthic diet) (Robinson and Wilson 1994).

To achieve a lower growth rate than the normal phenotype, dwarf fish must consume less food and (or) allocate a larger fraction of their energy budget to metabolism. The energy budget of dwarf fish has never been determined under natural conditions, probably due to the difficulty in estimating food consumption rates of fish using traditional methods based on stomach contents. These methods require the sacrificing of a large number of fish, necessitate a tremendous sampling effort in the field (Trudel et al. 2000), and may not be suitable for comparing the energy budget of sympatric populations of dwarf and normal fish.

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The objectives of this study were to compare the energy budget of dwarf and normal fish. We tested the hypotheses that dwarf fish have (*i*) lower food consumption rates and (*ii*) higher metabolic rates than normal individuals. This study was performed on two sympatric populations of dwarf lake whitefish (*Coregonus clupeaformis*) (DLW) and normal lake whitefish (NLW) located in northern Quebec. Sympatric populations of DLW and NLW have been observed in several lakes in North America and Europe (Svärdson 1970; Pigeon et al. 1997). DLW rarely exceed 250 mm and mature at a smaller size and at an earlier age than NLW (Fortin and Gendron 1990; Bodaly et al. 1991). NLW feed on zooplankton when they are small and shift to benthic invertebrates when they reach a size of about 200–250 mm, while DLW mainly consume zooplankton throughout their life (Scott and Crossman 1973; Doyon et al. 1998*a*).

DLW are usually not found when cisco (*Coregonus artedi*) are present, suggesting that these fish may occupy the same ecological niche (Pigeon et al. 1997; Doyon et al. 1998*b*). Similar to that of DLW, the diet of cisco mainly consists of zooplankton (Scott and Crossman 1973). Cisco also tend to be smaller and mature earlier than NLW (Scott and Crossman 1973). Hence, we also compared the energy budget of two sympatric populations of cisco and NLW in this study. Although DLW and cisco are genetically distinct species, they were classified into the same functional group (i.e., dwarf phenotype) for the purpose of this study.

Food consumption rates of DLW, NLW, and cisco were estimated using a mercury (Hg) mass balance model that we recently developed (Trudel et al. 2000) as well as a radioactive cesium (^{137}Cs) mass balance model refined by Rowan and Rasmussen (1996). Mass balance models of chemical tracers like Hg and ^{137}Cs offer an alternative to stomach contents for accurately estimating food consumption rates of fish (Rowan and Rasmussen 1996; Trudel et al. 2000). This approach requires considerably less sampling effort than stomach contents and has recently been validated for Hg by Trudel et al. (2000).

Methods

Species and study sites

This study was conducted on lake whitefish from the Caniapiscau Reservoir, Lake Serigny, Lake Rond-de-Poêle, and the Ottawa River and on cisco from Lake Rond-de-Poêle and the Ottawa River. NLW are present in these four systems, while DLW are only present in the Caniapiscau Reservoir and Lake Serigny (Appendices A and B). The Caniapiscau Reservoir is located in northern Quebec (54°00′N, 69°52′W) and was impounded between 1981 and 1984 (Doyon et al. 1998*b*). It is the largest reservoir of the hydroelectric complex of La Grande River. Lake Serigny (55°18′N, 69°42′W) and Lake Rond-de-Poêle (52°26′N, 77°00′W) are also located in northern Quebec and served as reference sites for evaluating the effects of the impoundment of the La Grande complex. Finally, the Ottawa River is located on the border of Quebec and Ontario and flows into the St. Lawrence River near Montreal (Quebec).

Food consumption and growth rates

Food consumption rates (*I*, grams per gram per day) of DLW, NLW, and cisco from the Caniapiscau Reservoir and the two lakes located in northern Quebec were estimated with a Hg mass balance model as (Trudel et al. 2000)

$$(1)\qquad I = \frac{C_{t+\Delta t} - C_t e^{-(E+G+K)\Delta t}}{\alpha \cdot C_d \cdot [1 - e^{-(E+G+K)\Delta t}]}(E+G+K)$$

where C_t and $C_{t+\Delta t}$ are the concentrations of Hg in fish at times t and $t + \Delta t$ (micrograms per gram), respectively, Δt is the time interval (days), α is the assimilation efficiency of Hg from food (dimensionless), C_d is the concentration of Hg in food (micrograms per gram), E is the elimination rate of Hg (micrograms per microgram per day), G is the specific growth rate (grams per gram per day), and K is the loss of Hg due to spawning (micrograms per microgram per day). This model has been successfully validated and provides food consumption rates similar to those obtained using stomach contents and a mass balance model of radiocesium (Trudel et al. 2000).

The assimilation efficiency of Hg from food was set to 0.8 (Norstrom et al. 1976) (Table 1). The elimination rate of Hg from fish was estimated using fish size (W, grams) and water temperature (T, degrees Celsius) as (Trudel and Rasmussen 1997)

$$(2)\qquad E = \varphi \cdot W^{\beta} \cdot e^{\gamma T}$$

where φ, β, and γ are empirically derived constants (Table 1).

The loss rate of Hg due to spawning was estimated as (Trudel et al. 2000)

$$(3)\qquad K = \frac{Q \cdot \mathrm{GSI}}{365}$$

where Q is the ratio of Hg concentration in the gonads to that in fish, GSI is the gonadosomatic index, and 365 represents the number of days in a year.

To estimate the feeding rate of DLW, NLW, and cisco with the Hg mass balance model, we obtained the age, mass, and Hg concentration of lake whitefish and cisco and Hg concentration in their food from Doyon et al. (1996, 1998*a*) and Hydro-Québec databases. The gonadosomatic indices of lake whitefish and cisco were taken from Rowan and Rasmussen (1996). Mercury concentration and fish size were modeled as a function of age using linear and nonlinear regressions (Trudel et al. 2000). Daily water temperature of the Caniapiscau Reservoir, Lake Serigny, and Lake Rond-de-Poêle was modeled with a Gaussian function as

$$(4)\qquad T = 3.8 + 9.5e^{\frac{-(JD-223)^2}{61^2}}$$

where JD is the Julian day. Specific growth rate was estimated as (Ricker 1979)

$$(5)\qquad G = \frac{1}{\Delta t}\ln\left(\frac{W_{t+\Delta t}}{W_t}\right)$$

where W_t and $W_{t+\Delta t}$ are fish masses at times t and $t + \Delta t$ (grams). Specific growth rate was estimated for each age-class using the mass of two consecutive age-classes. Food consumption rates were estimated on a daily basis by interpolating fish size and Hg concentration between two adjacent age-classes (Trudel et al. 2000). Annual food consumption rates were then determined by summing the daily ration values obtained during these intervals.

Food consumption rates of NLW and cisco from the Ottawa River were estimated using the ^{137}Cs mass balance model outlined in Rowan and Rasmussen (1996). Fish age and mass, ^{137}Cs concentration in fish and their food, and water temperature were obtained from Rowan and Rasmussen (1996) and Rowan et al. (1997). Fish mass and ^{137}Cs concentration were modeled as a function of fish age using linear and nonlinear regressions. The elimination rate of ^{137}Cs from fish was estimated using fish size and water temperature (Rowan and Rasmussen 1995).

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Table 1. Parameters of the Hg mass balance model.

Symbol	Parameter description	Value	Source[a]
α	Assimilation efficiency	0.80	1
φ	Coefficient of Hg elimination	0.0029	2
β	Allometric exponent of Hg elimination	–0.20	2
γ	Temperature coefficient of Hg elimination	0.066	2
Q_m	Ratio of Hg concentration in the gonads and whole fish for males	0.59	3
Q_f	Ratio of Hg concentration in the gonads and whole fish for females	0.12	3

[a]1, Norstrom et al. (1976); 2, Trudel and Rasmussen (1997); 3, Trudel et al. (2000).

Allocation of energy to growth

The relationship between growth and consumption rates was examined using simple and multiple regression analyses (Sokal and Rohlf 1995). In this study, growth rates represented the sum of somatic (P_s, joules per day) and gonad growth (P_g, joules per day). Somatic growth was estimated as

$$(6) \qquad P_s = G \cdot W \cdot E_f$$

where E_f is the energy density of fish (joules per gram). Gonad growth was estimated in adult fish as

$$(7) \qquad P_g = \mathrm{GSI} \cdot W \cdot E_g$$

where E_g is the energy density of the gonads (joules per gram). Energy density is negatively correlated with water content in fish (Hartman and Brandt 1995). Unfortunately, we did not measure either the energy density or the water contents of the fish examined in our study and had to rely on a different strategy to estimate their energy density. In their paper on the bioenergetics of Lake Michigan bloater (*Coregonus hoyi*) (a species closely related to cisco and lake whitefish), Rudstam et al. (1994) developed an empirical model of energy density using water content as the predictor that was valid for cisco, bloater, and lake whitefish. They then developed an empirical model of water content for bloater using body size as the independent variable. Finally, they combined these two regression models to provide a relationship between energy density and fish size. The later equation was used in our study to estimate the energy density of cisco and lake whitefish, as the only information we could use to estimate the energy density of these fish was their size. We used this equation, as no such model has been derived specifically for cisco and lake whitefish and because the energy content of these three coregonine species is similar (Rudstam et al. 1994). The energy densities of testis and ovary were assumed to be 20% higher than the energy density of the soma (Diana 1983).

The proportion of the energy budget allocated to growth (both somatic and gonad growth) was estimated for each age-class as

$$(8) \qquad \mathrm{CE} = \frac{P_s + P_g}{\mathrm{DR}}$$

where CE is the gross conversion efficiency and DR is daily ration of fish (joules per day). Daily ration was obtained by converting food consumption rate estimated with eq. 1 (grams per gram per day) to joules per day assuming that the energy density of the food consumed by lake whitefish and cisco was equal to 3350 J·g wet mass^{-1} (Trudel et al. 2000).

Allocation of energy to metabolism

The energy budget of a fish can be written as

$$(9) \qquad \mathrm{DR} = P_s + P_g + R_T + F + U$$

where R_T is the total respiration or metabolic rate (joules per day) and F and U are the feces and excretory products (joules per day), respectively. Thus, R_T can be determined by difference, provided that the other parameters of the budget are known, as

$$(10) \qquad R_T = \mathrm{DR} - (P_s + P_g + F + U)$$

Parameters F and U were derived from the bioenergetic model of Rudstam et al. (1994) to estimate R_T of DLW, NLW, and cisco. The proportion of the energy budget allocated to R_T was estimated by dividing R_T by daily ration.

Statistical analyses

Food consumption rates, conversion efficiency, and metabolic rates were compared between DLW, cisco, and NLW using a 2 × 4 analysis of variance (ANOVA) (Sokal and Rohlf 1995), where 2 is the number of phenotypes (dwarf and normal) and 4 is the number of sites being compared. For these analyses, DLW and cisco were assigned to the dwarf phenotype, while NLW were assigned to the normal phenotype. Independent linear contrasts were used to interpret the significant interaction between phenotype and site (Sokal and Rohlf 1995).

Food consumption rates were compared between fish on a relative basis (i.e., grams per gram per day) in this study, as body size varied between phenotypes. The use of specific feeding rates to compare the energy budget of fish has been criticized as food consumption rates tend to scale to body size with an allometric exponent around 0.8 (Hewett and Kraft 1993). It has been suggested that feeding rates could be appropriately compared between fish of different size if they were divided by $W^{0.8}$ instead of W (Hewett and Kraft 1993). However, this rule of thumb is only valid if the allometric exponent of food consumption rates is equal to 0.8 and should not be applied without first examining the validity of this assumption. The allometric exponent of food consumption rates is not significantly different from isometry in lake whitefish (Trudel et al. 2000). Thus, food consumption rates of lake whitefish can be appropriately compared across size if they are divided by W and not by $W^{0.8}$. This indicates that specific feeding rates can be used in this study to compare the energy budget of DLW, cisco, and NLW. While this approach is satisfactory for the populations examined in this study, we also performed an analysis of covariance (ANCOVA) on absolute feeding rates using body size as a covariate and phenotype as a categorical variable, since the ANCOVA is usually considered to be the best procedure for removing the effects of body size on allometric relationships (Sokal and Rohlf 1995).

Because adult fish tend to have higher metabolic rates than juvenile fish (Rowan and Rasmussen 1996), it is important to control for the potential effects of maturation status when the energy budgets of the dwarf and normal phenotypes are compared. In this study, 78 and 60% of the age-classes were represented by adult fish in the dwarf and normal phenotypes, respectively (Appendices A and B). Unfortunately, it was not possible to perform a three-way ANOVA with phenotypes, sites, and maturation status as factors, as juvenile fish from the dwarf phenotype were represented by only one age-class (Appendices A and B). Hence, this analysis would have been highly unbalanced, making the results from the ANOVA difficult to interpret (Sokal and Rohlf 1995). Instead of an ANOVA, we controlled for maturation status by using a Wilcoxon

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sign rank test (Sokal and Rohlf 1995). The dwarf and normal phenotypes were paired by maturation status within each site. This analysis was performed on the average values of food consumption rates, conversion efficiency, and metabolic costs and allowed us to determine if there was a consistent effect of phenotypes when we controlled for the effects of maturation status. We also performed a Wilcoxon sign rank test to determine if there was a consistent difference in the energy budget of adult and juvenile fish. For this analysis, adult and juvenile fish were paired by phenotypes within a site. Since two statistical tests were performed with the same data set, the significance level of the Wilcoxon sign rank test was adjusted using a Bonferroni correction (Sokal and Rohlf 1995). This was achieved by dividing the significance level by 2 (i.e., $\alpha' = 0.05/2 = 0.025$).

Results

Food consumption and growth rates

At a given age, NLW were larger than both DLW and cisco at the sites examined in this study (Fig. 1). DLW and cisco matured earlier and at a smaller size and had a shorter life span than NLW in these sites (Table 2).

Food consumption rates estimated with the Hg and ^{137}Cs mass balance models averaged 0.032 $g \cdot g^{-1} \cdot day^{-1}$ in DLW, 0.021 $g \cdot g^{-1} \cdot day^{-1}$ in NLW, and 0.028 $g \cdot g^{-1} \cdot day^{-1}$ in cisco (Fig. 2). Despite their lower growth rate, DLW and cisco tended to consume approximately 40–50% more food than NLW (Fig. 2). However, the feeding rate differences between cisco and NLW varied between sites: cisco consumed 69 and 16% more food than NLW in Lake Rond-de-Poêle and the Ottawa River, respectively (Fig. 2). Food consumption rates varied significantly between phenotypes and among sites (Table 3). The ANCOVA also indicated that food consumption rates of DLW and cisco were significantly higher than those of NLW ($F_{1,50} = 19.8$, $p < 0.0001$). The interaction between phenotype and site was also significant (Table 3). Linear contrasts indicated that the difference between cisco and NLW was not constant between sites ($F_{1,45} = 6.8$, $p < 0.02$). They also indicated that the difference between DLW and NLW was not significantly different from the difference between cisco and NLW ($F_{1,45} = 0.2$, $p > 0.6$).

The dwarf phenotype consistently consumed more food than the normal phenotype when we controlled for maturation status ($T_8 = 0$, $p < 0.01$) (Table 4). Adult fish tended to consume more food than juvenile fish in the dwarf phenotype but not in the normal phenotype (Table 4). The maturation status was not significant when we controlled for the phenotype effect and when the significance level was Bonferroni corrected ($T_8 = 3$, $0.025 < p < 0.05$).

Allocation of energy to growth

Growth rates tended to increase with the quantity of food consumed by lake whitefish and cisco (Fig. 3). At a given consumption rate, growth rates tended to be higher in NLW, lower in DLW, and intermediate in cisco (Fig. 3). The best empirical model predicting the growth rate (P, joules per day) of coregonines in these systems was (SE in parentheses)

$$(11) \quad \log_{10} P = -0.58(0.22) + 0.94(0.06) \log_{10}\mathrm{DR} - 0.56(0.07)\ \mathrm{DW} - 0.28(0.07)\ \mathrm{CIS}$$

$$R^2 = 0.94,\ \mathrm{SE}_{est} = 0.158,\ n = 53,\ p < 0.0001.$$

where DW is a binary variable that takes the value of 1 for DLW and 0 for NLW and cisco and CIS is a binary variable that takes the value of 1 for cisco and 0 for the other species. This model indicates that DLW and cisco allocated 3.6- and 1.9-fold less energy to growth, respectively, than NLW. Cisco tended to allocate a larger fraction of their energy budget to growth than DLW. However, this may represent a site effect, as fish from the Ottawa River had higher conversion efficiencies (Fig. 4).

Conversion efficiency averaged 4.8% in DLW, 16.0% in NLW, and 9.5% in cisco (Fig. 4). Conversion efficiency tended to be higher in fish from the Ottawa River than in those from the sites located in northern Quebec (Fig. 4). Conversion efficiency varied significantly between phenotypes and among sites (Table 3). The interaction between phenotype and site was not significant (Table 3).

The dwarf phenotype consistently had lower conversion efficiency than the normal phenotype when we controlled for maturation status ($T_8 = 0$, $p < 0.01$) (Table 4). Conversion efficiency was not consistently lower in adult than in juvenile fish (Table 4). The maturation status was not significant when we controlled for the phenotype effect ($T_8 = 14$, $p > 0.6$).

Allocation of energy to metabolism

DLW and cisco tended to spend more energy in respiration than NLW (Fig. 5). The proportion of the energy budget allocated to R_T averaged 62.7% in DLW, 51.5% in NLW, and 58.0% in cisco (Fig. 5). Coregonines from the Ottawa River tended to allocate a lower fraction of their energy budget to R_T than fish from northern Quebec (Fig. 5). The R_T varied significantly between phenotypes and among sites (Table 3). The interaction between phenotype and site was not significant (Table 3).

The dwarf phenotype consistently had higher metabolic costs than the normal phenotype when we controlled for maturation status ($T_8 = 0$, $p < 0.001$) (Table 4). Metabolic costs were not consistently lower in adult than in juvenile fish (Table 4). The maturation status was not significant when we controlled for the phenotype effect ($T_8 = 14$, $p > 0.6$).

Discussion

Bioenergetics of polymorphism

The analyses performed in this study showed that specific feeding rates of DLW and cisco were, on average, about 40–50% higher than those of NLW. Furthermore, we showed that the conversion efficiency of DLW and cisco was two to three times lower than that of NLW. Therefore, the low growth of dwarf fish can be more readily explained in terms of high energy allocation to metabolism rather than by a low rate of food consumption. In this study, DLW and NLW allocated about 60 and 50% of their energy budget to metabolism, respectively. Although this difference appears to be small, fish growth is quite sensitive to small variations associated with metabolic rates (Kitchell et al. 1977). Thus, even if only a small fraction of the energy budget is diverted to metabolism, it can have large effects on growth.

It could be argued that food consumption rates of DLW and cisco were larger than those of NLW because specific feeding rates are generally higher in smaller fish (Hewett

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Fig. 1. Total length of DLW (solid circles), NLW (open circles), and cisco (triangles) from (*a*) the Caniapiscau Reservoir, (*b*) Lake Sérigny, (*c*) Lake Rond-de-Poêle, and (*d*) the Ottawa River in relation to age. The line represents the von Bertalanffy growth curve and was fitted by nonlinear regression. For the Ottawa River, a power equation was used to model fish length.

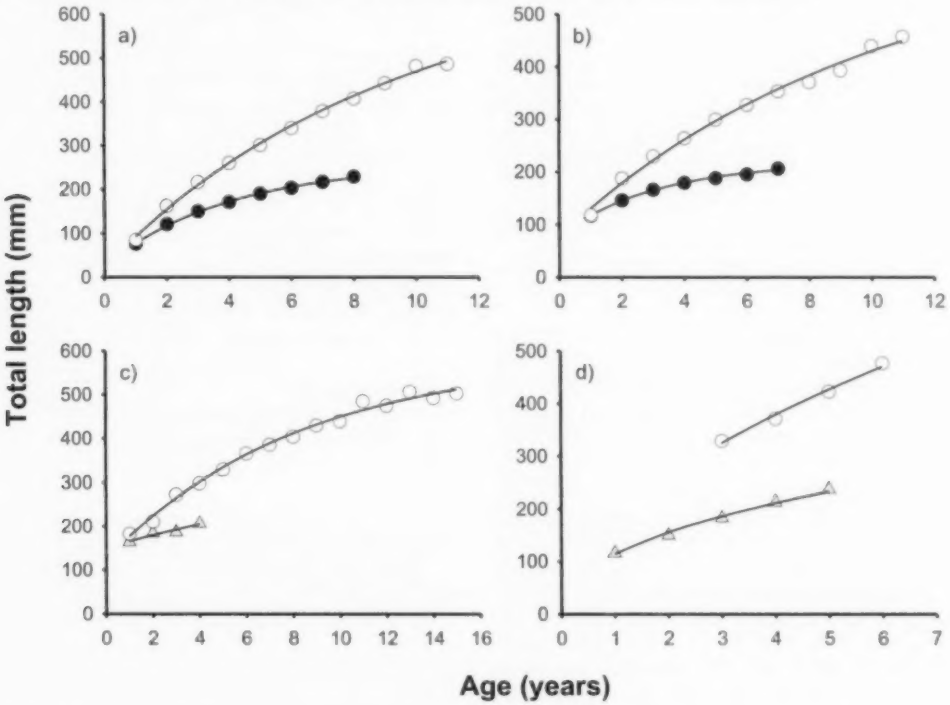

Table 2. Life history parameters of DLW, NLW, and cisco from four aquatic ecosystems.

Population	Age at maturity (years)	Length at maturity (mm)	Longevity (years)
Caniapiscau Reservoir			
DLW	2–3	148	<10
NLW	6	345	>15
Lake Serigny			
DLW	3	165	<10
NLW	6–7	329	>15
Lake Rond-de-Poêle			
Cisco	2	180	<10
NLW	7	388	>15
Ottawa River			
Cisco	2	155	na
NLW	4	378	na

Note: na, not available.

and Kraft 1993). This is unlikely to be the case in this study, since the allometric exponent of feeding rates is not significantly different than 1.0 in lake whitefish (Trudel et al. 2000). In addition, similar results were obtained by controlling for the effects of body size using an ANCOVA. Thus, DLW and cisco did not consume proportionally more food than NLW simply because they were smaller.

Rowan and Rasmussen (1996) recently showed that metabolic costs tended to be higher in adult than in juvenile fish. Hence, it may be argued that metabolic rates were higher in DLW and cisco simply because DLW were mostly represented by adult fish (Appendices A and B). In this study, there were no consistent patterns in conversion efficiencies or metabolic rates and maturation status in DLW, NLW, and cisco. In addition, when we controlled for the effects of maturation status, DLW and cisco consistently consumed more food, had lower conversion efficiencies, and allocated a larger fraction of their energy budget to metabolism than NLW. It is also important to note that these results are not unique to lake whitefish. Conversion efficiencies and metabolic costs were similar in juvenile and adult brook trout (*Salvelinus fontinalis*) from a small stream in Quebec (Tucker and Rasmussen 1999). Thus, the higher metabolic rates of the dwarf phenotype observed in this study cannot be attributed to differences in maturation status.

The proportion of the energy budget allocated to metabolism differed between dwarf and normal phenotypes because either standard metabolic rates (SMR), activity costs, or both were higher in DLW and cisco than in NLW. Activity costs tend to be positively correlated with feeding rates in actively foraging fish (Kerr 1982). Thus, since DLW and cisco eat about 40–50% more food than NLW, they are also likely to be more active. We may ask, however, why would DLW and cisco consume more food and spend more energy in foraging activity if they cannot grow as fast or even faster than NLW? The parsimonious interpretation is that DLW and cisco also have higher SMR than NLW. Hence, if SMR were higher in DLW and cisco, they would need to consume more

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Fig. 2. Average food consumption rates of DLW (solid bars), NLW (open bars), and cisco (shaded bars) from four sites. The error bars represent 1 SE; bw, body weight.

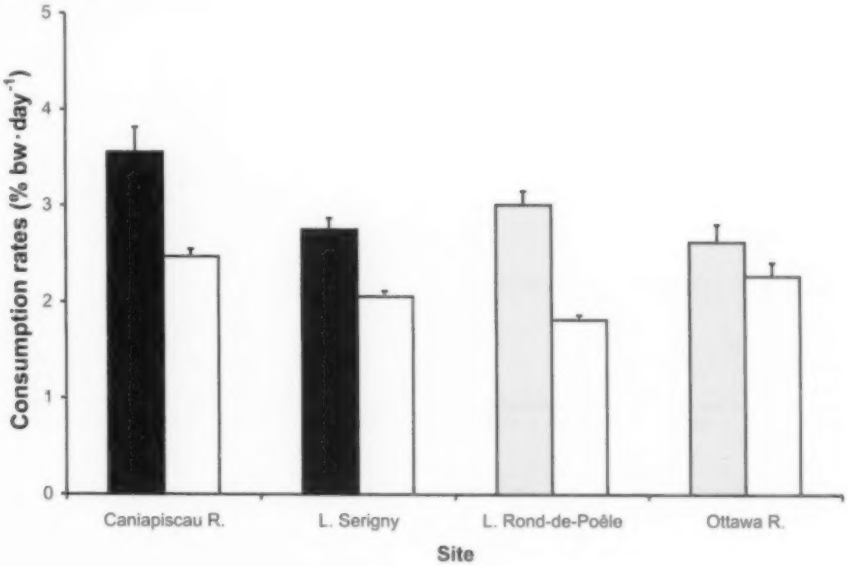

Table 3. Statistical comparison of the energy budget of DLW, NLW, and cisco (two-way ANOVA).

	F	df	*p*
Consumption rates ($g \cdot g^{-1} \cdot day^{-1}$)			
Phenotype	64.9	1,45	<0.0001
Site	11.8	3,45	<0.0001
Phenotype × site	2.9	3,45	<0.05
Conversion efficiency			
Phenotype	97.6	1,45	<0.0001
Site	6.2	3,45	<0.002
Phenotype × site	1.0	3,45	>0.4
Metabolic rates (% of feeding rates)			
Phenotype	97.1	1,45	<0.0001
Site	6.2	3,45	<0.002
Phenotype × site	1.0	3,45	>0.4

food to meet their energy requirements and would also need to spend more energy to acquire this food. Thus, both SMR and activity costs are expected to be higher in DLW and cisco.

SMR of fish have generally been estimated using the intercept of the relationship between swimming costs and swimming speed (Brett 1964). SMR have not yet been determined in DLW but have been estimated in NLW and a variety of cisco species. In general, the intercept of the relationship between swimming costs and swimming speed is 30–80% higher in cisco than in NLW (Rudstam et al. 1994), which supports our interpretation that the SMR of cisco are higher than those of NLW. In contrast, however, Bernatchez and Dodson (1985) compared swimming costs of cisco and NLW and concluded that SMR were lower in cisco. However, their interpretation is not supported by their data. In their study, they measured swimming costs of cisco and NLW swimming at speeds ranging from 20 to 55 $cm \cdot s^{-1}$ and from 20 to 40 $cm \cdot s^{-1}$, respectively. These experiments were performed at 5, 12, and 17°C for NLW and only at 12°C for cisco. Swimming costs of NLW swimming at speeds exceeding 40 $cm \cdot s^{-1}$ were estimated from the relationship between swimming costs and swimming speed derived for rainbow trout (*Oncorhynchus mykiss*) by Rao (1968). We reanalyzed the data presented by Bernatchez and Dodson (1985) in their fig. 1 by excluding the values that were estimated with the rainbow trout model. From their data, it is apparent that there is an interaction between swimming speed and water temperature, as the slope of the relationship between swimming costs and swimming speed appears to be lower for fish maintained at 17°C (Fig. 6). Also, the swimming costs of cisco and NLW maintained at 12°C were quite similar. The best empirical model predicting swimming costs explained about 96% of the variance and included swimming speed, water temperature, and the interaction between swimming speed and water temperature as independent variables. There was no significant difference between the swimming costs of cisco and NLW ($F_{1,12} = 0.01$, $p > 0.9$), suggesting that the SMR of cisco and NLW are similar. Thus, the laboratory evidence supporting the hypothesis that cisco have higher SMR than NLW is equivocal at present, and further work will be required to test this hypothesis.

It has been suggested that the biochemical activity of some enzymes involved in cell respiration and glycolysis could be used as surrogates of metabolic rates of fish in situ. In particular, Sullivan and Somero (1980) showed that the biochemical activity of lactate dehydrogenase was higher in more active fish. In addition, lactate dehydrogenase activity is positively correlated with swimming costs in yellow perch (*Perca flavescens*) (G. Sherwood, Department of Biology, McGill University, Montreal, Que., personal communication). Thus, the biochemical activity of lactate dehydrogenase may

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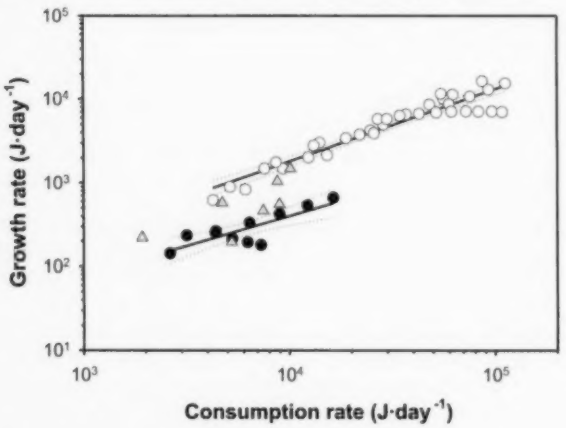
Table 4. Energy budget of the dwarf and normal phenotypes in relation to maturation status (immature or mature).

Site	Dwarf (immature)	Normal (immature)	Dwarf (mature)	Normal (mature)
Food consumption rates (% body weight·day^{-1})				
Caniapiscau River	2.77 (—)	2.33 (0.07)	3.71 (0.25)	2.68 (0.21)
Lake Serigny	2.52 (—)	2.14 (0.12)	2.81 (0.13)	1.99 (0.02)
Lake Rond-de-Poêle	2.73 (—)	1.87 (0.01)	3.16 (0.03)	1.78 (0.07)
Ottawa River	2.19 (—)	2.00 (—)	2.78 (0.16)	2.42 (0.06)
Conversion efficiency (% of food consumption rates)				
Caniapiscau River	5.4 (—)	18.3 (1.5)	4.8 (0.3)	14.3 (0.2)
Lake Serigny	7.4 (—)	15.1 (0.7)	3.9 (0.7)	17.6 (0.2)
Lake Rond-de-Poêle	3.7 (—)	18.5 (1.1)	6.2 (0.0)	12.5 (1.7)
Ottawa River	11.4 (—)	19.3 (—)	12.9 (0.9)	20.1 (1.1)
Metabolic costs (% of food consumption rates)				
Caniapiscau River	62.1 (—)	49.2 (1.5)	62.7 (0.3)	53.2 (0.2)
Lake Serigny	60.1 (—)	52.4 (0.7)	63.6 (0.8)	49.9 (0.2)
Lake Rond-de-Poêle	63.8 (—)	48.9 (1.1)	61.3 (0.0)	55.0 (1.7)
Ottawa River	56.1 (—)	48.2 (—)	54.5 (0.9)	47.4 (1.1)

Note: Standard errors are given in the parentheses. A dash indicates that the standard error could not be estimated, as sample size for these fish is one.

Fig. 3. Relationship between fish growth and consumption rates in DLW (solid circles), NLW (open circles), and cisco (shaded triangles).



be used as surrogate of fish swimming costs. Guderley et al. (1986) showed that the biochemical activity of lactate dehydrogenase was higher in cisco than in NLW. This suggests that swimming costs are higher in cisco, which is consistent with our interpretation.

Evolution of polymorphism in lake whitefish

Most polymorphic populations of fish consist of a limnetic phenotype that exploits zooplankton and a benthic phenotype that feeds on benthic invertebrates (Robinson and Wilson 1994). The limnetic phenotype tends to have longer and more gill rakers than the benthic phenotype, presumably to facilitate the retention and consumption of zooplankton (Robinson and Wilson 1994; Lu and Bernatchez 1999). Conversely, the benthic phenotype tends to have smaller and fewer gill rakers, possibly to retain only large particles and let the sediment go through the buccal cavity without clogging the respiratory surfaces. Hence, because of the morphology of their gill rakers, the limnetic phenotype is generally expected to be more efficient than the benthic phenotype when it is consuming plankton, while the benthic phenotype is expected to be more efficient than the limnetic phenotype when it is consuming benthic invertebrates (Schluter 1993, 1995). This hypothesis is supported by the work performed by Schluter (1993, 1995) on threespine stickleback (*Gasterosteus aculeatus*). The limnetic sticklebacks have more gill rakers than the benthic sticklebacks and feed almost exclusively on plankton (McPhail 1984). In his laboratory and field experiments, Schluter (1993, 1995) showed that limnetic sticklebacks consumed more food and grew faster than benthic sticklebacks when they were only fed with zooplankton. Conversely, he showed that benthic sticklebacks consumed more food and grew faster than limnetic sticklebacks when they were fed with benthic invertebrates. In addition, hybrids of limnetic and benthic sticklebacks had an intermediate response to these phenotypes. Hence, the different phenotypes of a polymorphic species appear to have evolved to efficiently exploit alternative resources (Schluter 1993, 1995).

In this study, the limnetic and benthic phenotypes were represented by DLW and NLW, respectively. Since DLW tend to have more gill rakers than NLW (Fenderson 1964; Lu and Bernatchez 1999), they were expected to be more efficient as planktivores than NLW (Bernatchez et al. 1999; Lu and Bernatchez 1999). DLW consumed more food than NLW when both phenotypes were consuming zooplankton. Based on these results, it may be tempting to conclude that DLW have evolved to become more efficient plankton feeders. The validity of this interpretation may be questionable, however. First, gill raker count usually differs by, on average, only one to four rakers and overlaps considerably between DLW and NLW (Fenderson 1964; Lu and Bernatchez 1999). This suggests that it is rather unlikely that DLW will retain substantially more and smaller plankton than NLW with their gill rakers. Unfortunately, there is not enough resolution in the dietary data of DLW and NLW at present to verify this hypothesis. Second, in some populations, DLW have actually less gill rakers than NLW (Fortin and Gendron 1990), suggesting that DLW could be even less efficient at capturing plankton than NLW. In particular, DLW from the

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Fig. 4. Proportion of the energy budget allocated to growth in NLW (open bars), DLW (solid bars), and cisco (shaded bars). The error bars represent 1 SE.

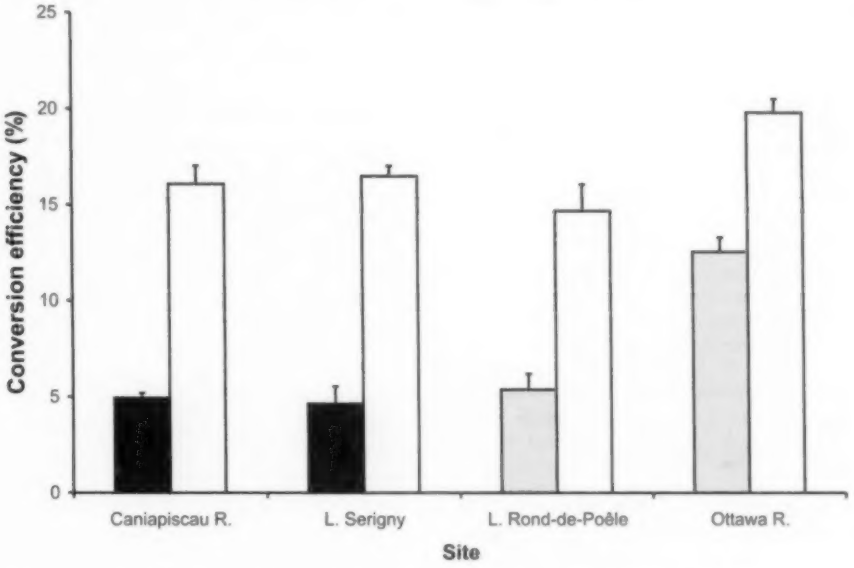
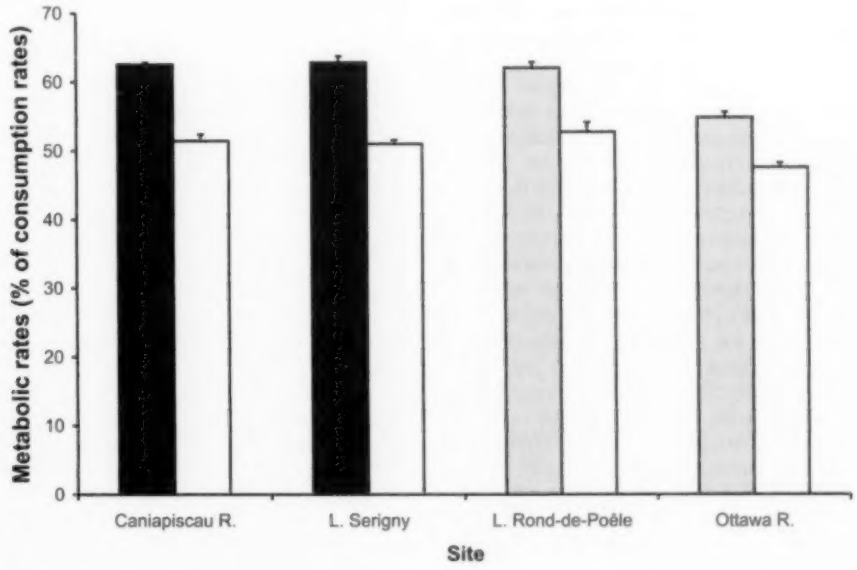


Fig. 5. Proportion of the energy budget allocated to respiration (R_T) in NLW (open bars), DLW (solid bars), and cisco (shaded bars). The error bars represent 1 SE.



Caniapiscau Reservoir, one of the studied populations, tend to have four less gill rakers than NLW (Gendron 1994). Third, if the number of gill rakers influences the capture efficiency of plankton in coregonine fish, we would expect cisco to eat more food than DLW and feeding rate differences between cisco and NLW to be larger than between DLW and NLW, since cisco tend to have 20–30 more gill rakers than NLW and DLW (Scott and Crossman 1973; Lindsey 1981). Yet, consumption rates were similar between cisco and DLW. In addition, feeding rate differences between cisco and NLW were similar to those obtained between DLW and NLW. Finally, growth and conversion efficiencies were much smaller for DLW than for NLW when they were feeding on zooplankton. Thus, it is unlikely that DLW has evolved to become an efficient planktivore. This also suggests that gill raker morphology does not necessarily predict trophic efficiency.

Instead, the results obtained in this study suggest that lake whitefish that were inefficient at converting food to somatic tissues (i.e., those with high metabolic rates) were probably

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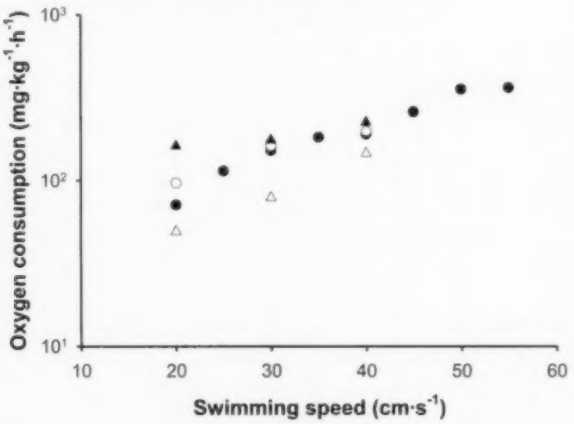
Fig. 6. Oxygen consumption of NLW held at 5°C (open triangles), 12°C (solid circles), and 17°C (solid triangles) and cisco held at 12°C (open circles) in relation to swimming speed. Data were extracted from Bernatchez and Dodson (1985).



not capable of reaching a large size and competing with NLW for benthic invertebrates. These fish were probably constrained to forage on zooplankton throughout their existence and then became the dwarf phenotype. This interpretation suggests that polymorphic populations of lake whitefish are likely to have evolved in systems where benthic production is lower than zooplankton production and in the absence of pelagic competitors like cisco. It is also based on the assumption that NLW is the ancestral form, and zooplankton biomass is not limiting to NLW but is limiting to DLW. These assumptions are indirectly supported by the literature. First, DLW are only found in sympatry with NLW, while allopatric populations of NLW exist (Pigeon et al. 1997). This suggests that the dwarf phenotype was derived from the normal phenotype (Pigeon et al. 1997). Second, NLW can shift their diet back to zooplankton in some lakes during the summer months, presumably because benthic invertebrates are scarce and zooplankton biomass is abundant in these lakes (Bernatchez et al. 1999). Third, DLW do not coexist with cisco, probably due to the similarity of these species and presumably because zooplankton biomass is not sufficient to sustain both DLW and cisco. Finally, increasing the production of the pelagic zone increases the biomass of DLW (Doyon et al. 1998*a*), suggesting that the abundance of DLW is limited by zooplankton production. However, although these observations support our interpretation that polymorphism in lake whitefish has evolved to reduce intraspecific competition for benthic invertebrates, it still remains to be tested by direct estimates of zooplankton and benthic invertebrate production in these systems.

Competition between cisco and DLW

DLW usually do not co-occur with cisco (Pigeon et al. 1997; Doyon et al. 1998*b*). Because DLW and cisco have a similar diet, morphology, and life history, they may occupy the same ecological niche and hence may compete for limited resources (Pigeon et al. 1997). In this study, we provide further evidence that DLW and cisco play a similar functional role in aquatic ecosystems. Food consumption rates of DLW and cisco were similar. In addition, the lack of a significant interaction between phenotype and site for conversion efficiency indicates that the response of DLW and cisco to the presence of NLW was similar. These results indicate that DLW and cisco are unlikely to coexist due to the similarity of their diet, life history, and bioenergetics.

Metabolic rates and life history

Life history models have been developed to understand and predict the extent of life history variation in animals and plants (e.g., Roff 1984). These models attempt to maximize fitness of organisms, often defined as the lifetime production of offspring, by optimizing trade-offs between growth, reproduction, and mortality. As mortality rates generally increase with growth rates in fish (Roff 1984), life history theory predicts that fast-growing individuals will mature earlier. In contrast with these predictions, DLW and cisco, despite their lower growth rate, matured earlier than NLW (Fortin and Gendron 1990; Bodaly et al. 1991; this study).

Roff (1992) argued that slow-growing fish could mature earlier than fast-growing fish if their mortality rates were higher. Since the life span of DLW and cisco tends to be shorter than that of NLW, this suggests that their mortality rates are also higher. This supports Roff's (1992) interpretation that the earlier maturation of dwarf fish may be due to their higher mortality rates. The mortality rate of DLW and cisco could be higher than that of NLW if small fish were preferentially selected by piscivorous fish. In addition, due to their smaller size, DLW and cisco are likely to be vulnerable to predation for a longer period of time than NLW. This is unlikely, however, as the most abundant piscivore in these systems is northern pike (*Esox lucius*). These fish tend to select large rather than small prey (Scott and Crosman 1973), suggesting that mortality due to predation is expected to be higher in NLW. Quantitative estimates of predation rates on these two phenotypes will be necessary to evaluate this hypothesis. Alternatively, the lower survival of DLW and cisco may represent a cost of reproduction as the energy devoted for reproduction is drained away from the soma (Bell 1980).

An additional, and perhaps more important, factor that would be expected to contribute to early maturation in DLW and cisco is that the gain in fecundity that can be obtained by delaying maturation is smaller than the risk of dying before reproducing in dwarf fish. Since fecundity generally increases with body size in fish, there may be an advantage in delaying maturation, as more offspring will be produced if fish reach larger sizes. However, when metabolic rates are elevated (or conversion efficiency is low), fish may not be able to channel enough energy into growth. Thus, if growth levels off, like in DLW and cisco, the small gain that can be obtained in fecundity by delaying maturation may be offset by a greater risk of mortality. In this situation, fish should mature and reproduce earlier. This interpretation is supported by our data, as fish that matured earlier in this study also had higher metabolic rates. Similar results were also obtained by Forseth et al. (1994) and Tucker and Rasmussen (1999): fish that had higher metabolic rates and lower conversion efficiencies within a cohort also matured earlier. This interpretation is also consistent with the life history model of Hutchings (1993), who suggested that fish would mature earlier if the growth of adult fish were small relative to the growth of juvenile fish (i.e., if growth levels off). This

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interpretation is also supported by the work of Fox (1994) and Bertschy and Fox (1999), who showed that mean age at maturity was inversely correlated with the ratio of adult to juvenile growth rate in pumpkinseed (*Lepomis gibbosus*). In addition, Bertschy and Fox (1999) also showed that it was not significantly correlated with age-specific mortality rate. Thus, the maturation of fish may be intimately linked to patterns of energy allocation in fish.

Few studies have attempted to link life history strategy of fish with patterns of energy acquisition and energy allocation. This is probably due to the difficulty in estimating food consumption rates of fish under natural conditions using traditional methods based on stomach contents. The analyses performed in this study were based on feeding rates of fish that were estimated using a Hg mass balance model that we recently developed and validated (Trudel et al. 2000) as well as a ^{137}Cs mass balance model that was recently refined by Rowan and Rasmussen (1996). Mercury and ^{137}Cs are globally dispersed due to their long residence time in the atmosphere and can be readily detected in the biota with modern instruments. Mass balance models of chemical tracers require considerably less sampling effort in the field than methods based on stomach contents. In addition, these mass balance models can be applied to archived fish. Thus, mass balance models of chemical tracers offer a promising alternative to stomach contents for studying the bioenergetics of fish under natural conditions and for examining how energy allocation influences life history strategies of fish.

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Appendix A

Table A1. Body mass, Hg concentration in fish and in their food, and food consumption rates of fish determined from the Hg mass balance model developed by Trudel et al. (2000).

Phenotype	n	Age (years)	W_t (g)	$W_{t+\Delta t}$ (g)	C_t (μg·g^{-1})	$C_{t+\Delta t}$ (μg·g^{-1})	C_d (μg·g^{-1})	I (g·g^{-1}·day^{-1})
Caniapiscau Reservoir								
NLW	14	2 i	17.2	39.6	0.316	0.316	0.060	0.0242
	11	3 i	39.6	71.6	0.316	0.316	0.060	0.0216
	18	4 i	71.6	113.4	0.316	0.316	0.050	0.0228
	19	5 i	113.4	289.0	0.316	0.316	0.040	0.0245
	16	6 m	289.0	476.5	0.316	0.316	0.032	0.0296
	33	7 m	476.5	665.6	0.316	0.316	0.032	0.0274
	45	8 m	665.6	893.8	0.316	0.316	0.032	0.0255
	40	9 m	893.8	1163.5	0.316	0.316	0.032	0.0240
	25	10 m	1163.5	1477.0	0.316	0.316	0.032	0.0227
DLW	2	2 i	14.4	24.6	0.384	0.447	0.060	0.0277
	10	3 m	24.6	36.0	0.447	0.520	0.060	0.0308
	24	4 m	36.0	48.4	0.520	0.605	0.060	0.0333
	17	5 m	48.4	61.5	0.605	0.704	0.060	0.0364
	22	6 m	61.5	75.4	0.704	0.820	0.060	0.0403
	15	7 m	75.4	90.0	0.820	0.955	0.060	0.0448
Lake Serigny								
NLW	9	2 i	38.8	79.9	0.164	0.177	0.03	0.0242
	1	3 i	79.9	133.3	0.177	0.186	0.03	0.0223
	2	4 i	133.3	191.0	0.186	0.194	0.03	0.0204
	2	5 i	191.0	251.5	0.194	0.200	0.03	0.0187
	3	6 m	251.5	331.1	0.200	0.206	0.03	0.0204
	3	7 m	331.1	435.9	0.206	0.211	0.03	0.0203

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Table A1 (*concluded*).

Phenotype	n	Age (years)	W_t (g)	$W_{t+\Delta t}$ (g)	C_t (μg·g^{-1})	$C_{t+\Delta t}$ (μg·g^{-1})	C_d (μg·g^{-1})	I (g·g^{-1}·day^{-1})
	2	8 m	435.9	573.9	0.211	0.215	0.03	0.0199
	2	9 m	573.9	755.5	0.215	0.219	0.03	0.0196
	1	10 m	755.5	994.7	0.219	0.223	0.03	0.0194
DLW	2	2 i	19.6	35.1	0.168	0.195	0.03	0.0252
	6	3 m	35.1	45.0	0.195	0.225	0.03	0.0257
	9	4 m	45.0	50.6	0.225	0.260	0.03	0.0265
	10	5 m	50.6	53.8	0.260	0.301	0.03	0.0288
	5	6 m	53.8	55.7	0.301	0.348	0.03	0.0315
Lac Rond-de-Poêle								
NLW	3	1 i	48.5	89.4	0.063	0.069	0.015	0.0187
	6	2 i	89.4	148.7	0.069	0.076	0.015	0.0190
	7	3 i	148.7	227.0	0.076	0.083	0.015	0.0189
	8	4 i	227.0	322.8	0.083	0.091	0.015	0.0186
	8	5 i	322.8	432.7	0.091	0.100	0.015	0.0185
	2	6 m	432.7	552.1	0.100	0.110	0.021	0.0152
	7	7 m	552.1	676.2	0.110	0.121	0.021	0.0156
	7	8 m	676.2	800.5	0.121	0.133	0.021	0.0161
	5	9 m	800.5	921.1	0.133	0.146	0.021	0.0167
	3	10 m	921.1	1035.2	0.146	0.160	0.021	0.0173
	2	11 m	1035.2	1140.8	0.160	0.176	0.021	0.0183
	3	12 m	1140.8	1236.8	0.176	0.193	0.021	0.0192
	3	13 m	1236.8	1322.8	0.193	0.213	0.021	0.0206
	4	14 m	1322.8	1398.8	0.213	0.233	0.021	0.0216
Cisco	2	1 i	39.0	50.1	0.105	0.130	0.015	0.0273
	1	2 m	50.1	61.6	0.130	0.145	0.015	0.0313
	2	3 m	61.6	73.2	0.145	0.155	0.015	0.0318

Note: Fish size and Hg concentration were estimated by linear and nonlinear regression analyses. Data modified from Doyon et al. (1996, 1998) and Hydro-Québec databases. See Methods for a definition of the variables. i, immature; m, mature.

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Appendix B

Table B1. Body mass, ^{137}Cs concentration in fish and in their food, and food consumption rates of Ottawa River fish determined from the ^{137}Cs mass balance model recently refined by Rowan and Rasmussen (1996).

Species	n	Age (years)	W_t (g)	$W_{t+\Delta t}$ (g)	C_t (Bq·kg^{-1})	$C_{t+\Delta t}$ (Bq·kg^{-1})	C_d (Bq·kg^{-1})	I (g·g^{-1}·day^{-1})
Lake whitefish	1	3 i	326.0	487.0	6.82	7.70	1.836	0.0200
	1	4 m	487.0	752.7	7.70	8.58	1.836	0.0236
	1	5 m	752.7	1124.6	8.58	9.47	1.836	0.0247
Cisco	1	1 i	12.2	27.9	1.55	2.06	0.589	0.0219
	4	2 m	27.9	52.6	2.06	2.56	0.589	0.0274
	2	3 m	52.6	86.2	2.56	3.07	0.589	0.0307
	1	4 m	86.2	122.5	3.07	3.83	0.867	0.0253

Note: Fish size and ^{137}Cs concentration were estimated by linear and nonlinear regression analyses. Data modified from Rowan and Rasmussen (1996). See Methods for a definition of the variables. i, immature; m, mature.

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Nested analysis of genetic diversity in northwestern North American char, Dolly Varden (*Salvelinus malma*) and bull trout (*Salvelinus confluentus*)

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Abstract: Partitioning within-species genetic diversity is fundamental to conservation of the bioheritage, current viability, and evolutionary potential of individual taxa. We conducted a hierarchical analysis of genetic diversity in Dolly Varden (*Salvelinus malma*) and bull trout (*Salvelinus confluentus*) involving analysis of hybrid zones between Dolly Varden and bull trout, analysis of phylogenetic structure within species across their native ranges using mitochondrial DNA, and a microsatellite DNA survey of population subdivision of bull trout within single watersheds. Our analyses documented hybridization and some introgression between Dolly Varden and bull trout across a geographically widespread zone of secondary contact between the two species. Both species were subdivided into two major mtDNA lineages, and one lineage in Dolly Varden may have arisen through introgression with bull trout. Bull trout have low levels of microsatellite diversity within populations, but there was substantial interpopulation variation in allele frequencies. Allele frequency distributions suggested that recent, severe bottlenecks occur frequently in some bull trout populations. Our results illustrate partitioning of genetic variation at distinct levels of biological organization (species, phylogeographic lineages, local populations), and we address how such nested variation is fundamental to conservation of biodiversity.

Résumé : La compartimentation de la diversité génétique intraspécifique est une opération essentielle pour la conservation de l'héritage biologique, de la viabilité actuelle et du potentiel évolutif de taxons particuliers. Nous avons procédé à une analyse hiérarchique de la diversité génétique de la Dolly Varden (*Salvelinus malma*) et de l'Omble à tête plate (*Salvelinus confluentus*) qui comprend une étude des zones d'hybrides entre les deux espèces, une analyse basée sur l'ADN mitochondrial de la structure phylogénétique des espèces sur toute leur répartition géographique d'origine et un inventaire des microsatellites de l'ADN dans les subdivisions de populations de l'Omble à tête plate à l'intérieur de bassins hydrographiques particuliers. Nos analyses révèlent l'existence d'hybridation et d'introgression dans une large zone géographique de contact secondaire entre les deux espèces. Chacune des espèces se divise en deux lignées principales d'après son ADNmt; l'une des lignées de la Dolly Varden est peut-être apparue par introgression avec l'Omble à tête plate. L'Omble à tête plate possède une faible diversité des microsatellites à l'intérieur des populations, mais il y a une importante variation des fréquences d'allèles entre les populations. Les distributions de fréquence des allèles laissent croire que d'importants goulots d'étranglement récents se sont produits à plusieurs reprises dans quelques populations d'Omble à tête plate. Notre étude est un exemple de la compartimentation de la variation génétique à plusieurs niveaux distincts de l'organisation biologique (espèce, lignées phylogénétiques, populations locales); nous discutons de la pertinence fondamentale d'une telle variation emboîtée pour la conservation de la biodiversité.

[Traduit par la Rédaction]

Introduction

Biological diversity is fundamentally hierarchical in nature, and the binomial nomenclature system developed by Linneaus is the most common application of a formal hierarchical system of biodiversity (Mayr 1982). Two issues related to current taxonomic practices, however, continue to generate controversy: the nature and origin of species and recognition of biodiversity below the species level. First, species concepts and mechanisms of speciation have been a

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continual source of debate in evolutionary biology (Taylor 1999), and these uncertainties and the status of individual taxa as valid species remain lively aspects of evolutionary research (e.g., Howard and Berlocher 1998). Second, the last 20 years have seen a heightened awareness of the presence of evolutionary divergence within species and its importance to conservation of particular species (Utter 1981; Avise 1994). The subspecies category is the most formal recognition of such variability, but other nontaxonomic (yet only slightly less controversial) designations such as "evolutionary significant units" (ESU) (Waples 1995) have gained rapid acceptance in the conservation literature (e.g., Moritz 1994). Molecular assays of biodiversity have been instrumental in documenting the hierarchical structure of intraspecific variation (Avise 1994; Bernatchez 1995). This is particularly true for freshwater fishes where the often dendritic nature of stream geomorphology promotes development of a similar hierarchical pattern at the genetic level (e.g., Vrijenhoek et al. 1985; Meffe and Carroll 1994).

Char, fishes of the genus *Salvelinus*, have provided evolutionary biologists with rich material to investigate the nature and origin of biodiversity at and below the species level (e.g., McPhail 1961; Phillips et al. 1999). This Holarctic genus consists of at least 11 well-recognized species, but the exact composition of the genus and the systematic relationships of species have a long history of uncertainty (e.g., Behnke 1980). For instance, Dolly Varden (*Salvelinus malma*) and bull trout (*Salvelinus confluentus*) have largely parapatric distributions in northwestern North America. Dolly Varden are coastal in distribution from Washington State to western Alaska and east to the Mackenzie River (Haas and McPhail 1991). Bull trout tend to be more inland in distribution and are found from Nevada to Yukon Territory (see fig. 1 in Baxter et al. 1997). A few areas of sympatry have been identified using morphological and biochemical/molecular analyses and appear to be confined to coastal regions of large rivers (e.g., lower Fraser River) and in and around the Coast/Cascade Mountain divide and the Olympic Peninsula (Cavender 1978; Baxter et al. 1997; Leary and Allendorf 1997). The status of Dolly Varden and bull trout as distinct species, however, has been uncertain, and at one time, bull trout were considered conspecific with Dolly Varden (e.g., McPhail 1961). More recent morphological (Cavender 1978; Haas and McPhail 1991) and molecular phylogenetic data (e.g., Phillips et al. 1995) have supported their status as distinct species. Genetic evidence of the two char maintaining themselves as distinct gene pools in sympatry (Baxter et al. 1997; Leary and Allendorf 1997) has provided the most direct evidence that the two char are distinct biological species. We know little, however, about the extent of contact between Dolly Varden and bull trout and about patterns of hybridization and gene flow on both a large and small geographic scale.

In addition to the species status of individual taxa, documenting the depth and phylogenetic relationships of evolutionary lineages within species is important for phylogeographic inference (Avise 1994). Further, the nature and origins of phylogenetic divisions within species are considered an important step in setting conservation priorities (Moritz 1994; Bernatchez 1995). Deeper (and older) lineages may represent the most important components of a species' "bioheritage" because they may be less replaceable than more recent divergences (Bowen 2000). We have, however, only recently gained some understanding of such diversity in bull trout (Taylor et al. 1999), and what information does exist for Dolly Varden (e.g., Phillips et al. 1999) excludes large portions of its range.

Finally, the level of distinction among local populations within major lineages is also an important issue in understanding both the origin of diversity within individual species and the conservation of such diversity. For instance, there is a rich history of investigations of local population structure in salmonid fishes using biochemical and molecular markers (Ryman and Utter 1987; Allendorf and Waples 1996). Particular attention has been focussed on the use of such data to understand the relationship between heterozygosity and viability of individual populations as well as the level of gene flow and its relationship to demographic independence among local populations. Although some work has been completed on local population structure of bull trout in the United States (Leary et al. 1993; Spruell et al. 1999), there is little known about population structure and its implications for demography for either bull trout or Dolly Varden in Canada, which represents the heart of the range of both species.

In this paper, our primary goals were to further define the contact zones between Dolly Varden and bull trout in northwestern North America, resolve the extent of phylogenetic distinction within Dolly Varden to facilitate comparative analysis with bull trout, and test for population subdivision within phylogenetic lineages of bull trout to help define conservation units on a local basis. Our secondary goal was to illustrate how particular classes of molecular loci and assays are appropriate for specific questions at different levels of biological organization.

Materials and methods

General laboratory methods

Our analyses consisted of various polymerase chain reaction (PCR) based assays of regions of DNA that either were diagnostic for Dolly Varden or bull trout or resolved polymorphisms within either species. For these analyses, genomic DNA was extracted from fin clip samples that were stored in 95% ethanol. DNA was extracted using standard proteinase K – pronase tissue digestion and phenol–chloroform- or salt-based purification.

Hybridization between Dolly Varden and bull trout

We surveyed geographic areas for evidence of sympatric populations and for the presence of interspecific hybrids using samples that where obtained opportunistically or as part of a detailed study of hybridization within a single watershed (Fig. 1). Sample localities were tested for the presence of either or both species and for hybrid individuals by examining introns or intergeneric spacers at three independently segregating loci (Table 1). Two of the three markers resolved size-based differences between the species that required no further analysis. The third locus, ITS-1, was subjected to restriction enzyme analysis as outlined in Baxter et al. (1997). All markers have been assayed on a number of individuals (N = 30–100) for each locus from throughout the geographic range of each species to confirm their diagnostic nature (Baxter et al. 1997; Redenbach 2000). PCR assays were run in either a Stratagene "Robocycler" or an MJ Research PCJ 100 thermal cycler using be-

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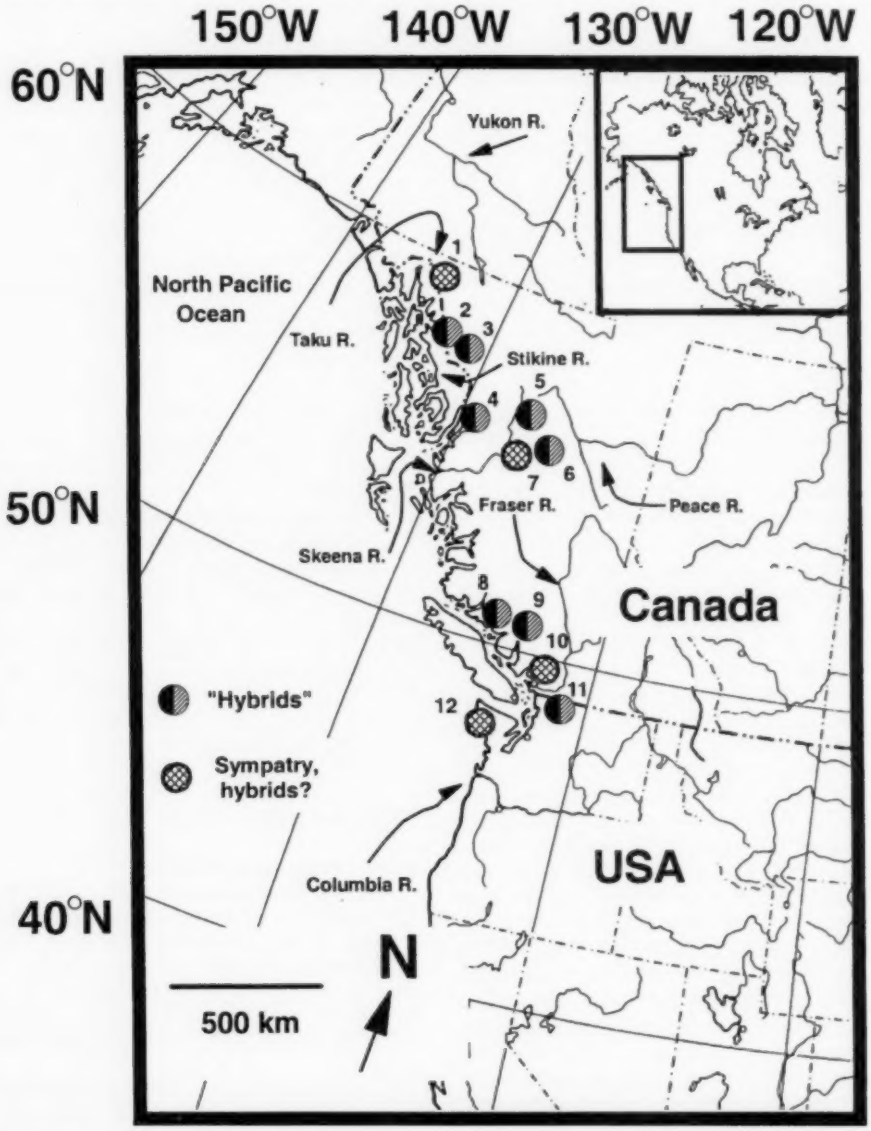
Fig. 1. Distribution of Dolly Varden, bull trout, and hybrids between these species based on molecular analyses at three nuclear loci. Also indicated are regions of sympatry established by molecular analyses but at which no hybrids have yet been detected. Sample codes are given in Table 5. Samples 11 and 12 represent the Skagit River and the East Fork Quinnalt River assayed by McPhail and Taylor (1995) and Leary and Allendorf (1997), respectively. Inset shows sample area in relation to North America.



tween 0.200 and 0.800 nM dNTPs and 0.5–1.0 unit of *Taq* polymerase per reaction.

We identified hybrids as fish of mixed ancestry. Using the diagnostic markers, we recognized hybrid genotypes as those individuals that were (*i*) heterozygous for species-specific fragments at all loci (putative F1 hybrids), (*ii*) heterozygous at one locus but homozygous for species-specific alleles at the other loci (putative backcrosses), or (*iii*) homozygous at all loci but for opposite specific-specific alleles at at least one of those loci (F1 × F1 or later-generation hybrids or backcrosses). Our analysis is conservative in that with three diagnostic markers, some fish classified as "pure" parental genotypes could actually be advanced-generation hybrids or backcrosses (see Avise 1994). At sites where parental and hybrid genotypes were detected ($N = 5$), we tested for differences in the percentages of hybrids using chi-square randomization tests (Roff and Bentzen 1989).

Phylogenetic groupings within species

For phylogenetic inference within both Dolly Varden and bull trout, we assayed sequence variation in the mtDNA NADH subunit 1 gene as outlined by Taylor et al. (1999). Sequences obtained from an extensive survey of variation in bull trout (Taylor et al. 1999) were combined with data collected from Dolly Varden from throughout their geographic range (Table 2). We also used ND1 sequence data collected from lake trout (*Salvelinus namaycush*) to serve as an outgroup taxon in our analyses. For Dolly Varden, our preliminary analyses indicated the presence of two divergent ND1

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Table 1. Primers, PCR conditions, species-specific diagnoses, and sources of molecular markers used in DNA analyses of Dolly Varden and bull trout.

Primer	Sequence	Cycles/annealing temperature (°C)	$MgCl_2$ (mM)	Allele size (basepairs)	Source
GH7	CTT ATG CAT GTC CTT CTT GAA			DV: 600	McKay et al. 1996
GH51	GTC AAG CTG ATA CAA CTC	40/50	1.5	BT: 540	
MTBF2	ATG CAC CAG TTG TAA GAA AG			DV: 520	J. Baker[b]
MTBR2	GTC GGA AGG ACA GCA GGG	40/56	1.5	BT: 700	Unpublished data
MD-1	CTT GAC TAT CTA GAG GAA GT			DV: 700	Phillips et al. 1995
ITS-R	AGC TTG CTG CGT TCT TCA TCG A	40/55	1.5	BT: 490,210[c]	
N1F	GGT ATG GGC CCG AAA GCT TA				Neilsen et al. 1998
N1R	GCC TCG CCT GTT TAC CAA AAA CAT	35/54	2	Clade A:340/200	
ND1C[a]	TGC AGC CGC TAT TAA GGG TTC G	na	na	Clade B:500/250[d]	Redenbach and Taylor 1999

Note: Loci are growth hormone (GH), metallothionein (MTB), internal transcribed spacer 1 (rDNA, ITS-1), and the mtDNA NADH-1 subunit (ND1). All sequences are written 5′–3′. na, not applicable.

[a]Used as sequencing primer.

[b]Fisheries Research Institute, University of Washington, P.O. Box 357908, Seattle, WA 98195-7980, U.S.A.

[c]RFLP difference resolved with *Sma* I.

[d]RFLP differences resolved with *Hae* III.

clades. The two clades could be distinguished by restriction site differences resolved with a number of enzymes (*Hae* III, *Hinf* I). We employed *Hae* III to resolve the presence of either or both clades in larger samples (up to $N = 10$) from throughout the range of Dolly Varden. As we were interested in the phylogeny of Dolly Varden mtDNA across the range of the species, we focussed our sampling on obtaining sequences from as wide an area as possible but did not sample extensively within populations. Phylogenetic analysis consisted of sequence divergence estimates (Kimura two-parameter) and maximum parsimony analyses. These analyses incorporated bootstrap resampling of sequence matrices ($N = 1000$) to assess the robustness of derived groupings. We also used maximum-likelihood analyses (Kishino and Hasegawa 1989) to statistically test for monophyletic groupings in combined analyses of Dolly Varden and bull trout sequences. All phylogenetic analyses were conduced using programs from the Phylip software package (Felsenstein 1993).

Population structure of bull trout

Samples were obtained during the summer of 1997 from Harrison Lake (Red Deer River, Banff National Park, Alta.) and Ice River (tributary of Kicking Horse River, upper Columbia River near Golden, B.C., in Yoho National Park). Bull trout were also sampled from two interior British Columbia watersheds: the upper Kootenay River watershed and a small portion of the lower Peace River, the Pine River watershed. The Kootenay River samples ranged from Ram Creek (Wigwam River) system near Elko, B.C., north to Skookumchuck Creek near Canal Flats, B.C. (about 200 km distant). The Pine River samples concentrated on two watersheds: the Sukunka and Murray rivers. Samples were collected in both 1997 (Kootenay and Sukunka rivers) and 1998 (Murray River) (Table 3).

We assayed microsatellite DNA variation using loci that had been isolated from other salmonid species (three loci) and also used two loci isolated from a bull trout genomic library (E.B. Taylor, unpublished data). PCR conditions followed standard procedures (Table 4), and all reactions were completed using 32-P end-labelled primers and PCR products were scored from autoradiographic films after polyacrlyamide electrophoresis (typically 6% Long Ranger gels).

Allelic variation within population samples was tested for departures from Hardy–Weinberg equilibrium as well as for departures from linkage equilibrium between loci. Pairwise tests of allele frequency differentiation between populations within watersheds were accomplished using Monte-Carlo resampling statistics. A variety of mutation-based and drift-based genetic distance algorithms are available for the calculation of population subdivision and genetic distances among samples. We considered drift-based methods of genetic subdivision to be the most appropriate. First, the postglacial origin of the freshwater populations of bull trout in our study areas sets their maximum age at about 12 000 years (Taylor et al. 1999). Over such short time frames, particularly when population histories may have involved large changes in population sizes, demographic processes probably overwhelm any postcolonization mutation-based differentiation patterns. Second, over such short evolutionary time periods, drift-based metrics alleles models tend to outperform alternatives based on mutation models (Paetkau et al. 1997), particularly in the range of the numbers of sample and loci employed in our study (Gaggiotti et al. 1999).

Consequently, we used methods based on the variance in allele frequencies (F_{st}) without regard to molecular size differences among alleles as a drift-based measure of genetic subdivision. Similar approaches have been adopted in other investigations of postglacial fish population genetics (e.g., Douglas et al. 1999). At each locus, we also calculated F_{st} (as estimated by θ) and its statistical significance based on allelic frequency differences using Genepop (Raymond and Rousset 1995) and Fstat (Goudet 1995). We also examined the level of genetic subdivision when samples were arranged hierarchically, i.e., genetic variation between watersheds (Peace versus Kootenay), variation among populations within watersheds, and variation within populations. This analysis was completed using the analysis of variance like design of Arlequin (Schneider et al. 1997), and the statistical significance of variance components was tested using randomization procedures in the Arlequin analysis.

In all statistical tests, the sequential Bonferroni procedure (Rice 1989) was followed to adjust the rates of Type I error over simultaneous tests to 0.05. Finally, we also utilized the microsatellite allele frequencies to test for evidence of recent bottlenecks in bull trout using the "mode-shift" indicator as implemented in Bottleneck (Piry et al. 1999). Populations that have undergone recent bottlenecks are expected to show a reduction in the proportion of low-frequency alleles relative to alleles of moderate abundance. Recent bottlenecks are those that have occurred within 40–80 generations, and the mode-shift test has resolved differences in empirical allele frequency distributions between bottlenecked and nonbottlenecked populations across a number of species (Luikart et al. 1998). The detection of recent bottlenecks in bull trout may be especially important, as populations so affected may not have had time to adapt to potential problems imposed by small population

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Table 2. Sample localities and sample sizes for Dolly Varden phylogeographic analyses.

Sample	Drainage	*N*	Northern clade	Southern clade
Udobnaya R.	Kuril Is., Russia	1/4	5	
Porozhistaya R.	Kuril Is., Russia	1/3	4	
Togiak L. (1)	Bristol Bay, Alaska	1/5	6	
Iliamna L. (2)	Bristol Bay, Alaska	1/5	6	
Klutina R. (3)	Copper R., Alaska	1/5	6	
Bonnet Plume R. (4)	Peel R., Yukon	/10	10	
Blackstone R. (5)	Peel R. Yukon	/5	5	
Tulsequah R. (6)	Taku R.	3/4	7	
Iskut R. (7)	Stikine R.	3/	3	
Zolzap R. (8)	Nass R.	/10	9	1
Ogden Channel (9)	Central coast B.C.	1/	1	
Noyes Sound (10)	Central coast B.C.	1/	1	
Ecstall R. (11)	Skeena R.	1/2	3	
Brent Cr. (12)	Queen Charlotte Is.	1/	1	
Aero R. (13)	Queen Charlotte Is.	1/	1	
Feather Cr. (14)	Queen Charlotte Is.	/2	2	
Honna R. (15)	Queen Charlotte Is.	1/	1	
Three Mile Cr. (16)	Queen Charlotte Is.	/1	1	
Ian R. (17)	Queen Charlotte Is.	/5	5	
Ain R. (18)	Queen Charlotte Is.	/5	5	
Kumealon Cr. (19)	Midcoast B.C.	1/	1	
Noosneck R. (20)	Midcoast B.C.	1/5	6	
Ocean Falls (21)	Midcoast B.C.	/11	11	
Dallery Cr. (22)	Midcoast B.C.	1/5	2	4
Wakeman R. (23)	South coast B.C.	/2		2
Southgate R. (24)	South coast B.C.	1/		1
Upper Deserted R. (25)	Midcoast B.C.	/4	4	
O'Connell L. (26)	Vancouver Is.	1/8	9	
Claninick R. (27)	Vancouver Is.	1/2	3	
Keogh R. (28)	Vancouver Is.	/10	5	5
Misty L. (29)	Vancouver Is.	1/	1	
Eve R. (30)	Vancouver Is.	/5		5
Jessie L. (31)	Vancouver Is.	/1		1
Thelwood Cr. (32)	Vancouver Is.	1/5	2	4
Phillips R. (33)	Vancouver Is.	1/5	1	5
Zeballos R. (34)	Vancouver Is.	2/2		4
Cowichan L. (35)	Vancouver Is.	1/4	5	
Mamquam R. (36)	South coast B.C.	1/		1
Mill Cr. (37)	South coast B.C.	1/3		4
Capilano R. (38)	South coast B.C.	/2		2
Seymour R. (39)	South coast B.C.	/2		2
Loon L. (40)	South coast B.C.	/8		8
Quinault R. (41)	Olympic Pen., Wash.	1/8		9
Dungeness R. (42)	Olympic Pen., Wash.	/5		5
South Fork Nooksack R. (43)	Puget Sound, Wash.	/5		5
Lake trout	Nation R. (upper Peace R., B.C.)	1/		

Note: Sample sizes separated by a solidus indicate the number sequenced left of the solidus and those assayed by restriction fragment length polymorphism analyses right of the solidus. Number codes following river and lake names denote sample localities given in Fig. 3.

sizes and may signal populations at risk of losing heteozygosity or variation at quantitative loci affecting fitness over the longer term (Luikart et al. 1998).

Results

Species distributions and hybridization

The geographic distribution of Dolly Varden and bull trout has traditionally been inferred from morphological analyses, and in several cases, sympatry was documented and hybridization suspected (e.g., Cavender 1978; Haas and McPhail 1991). We used diagnostic molecular markers to examine 12 localities from six drainage areas for the presence of both species and of interspecific hybrids, 10 of which had not been suspected as contact zones previously (Fig. 1; Table 5). Seven of the 12 sites contained both Dolly Varden and bull

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Table 3. Sample sizes (N), numbers of observed alleles (N_a), and expected (H_e) and observed (H_o) heterozygosities per locus and per bull trout population assayed.

Locus	HL	IR	SK	RC	CC	NBR	UBC	BR	Mean
Omy 77									
N	43	30	36	45	17	21	22	15	28.6
N_a	1	2	2	2	1	1	2	2	1.6
H_e	0	0.42	0.44	0.49	0	0.46	0.40	0.18	0.30
H_o	0	0.40	0.30	0.58	0	0.52	0.36	0.06	0.28
Sfo 18									
N	43	30	36	45	17	21	22	18	2.9
N_a	1	1	2	2	2	2	2	2	1.7
H_e	0	0	0.26	0.10	0.50	0.50	0.40	0.48	0.28
H_o	0	0	0.25	0.11	0.52	0.71	0.40	0.31	0.29
Ssa 197									
N	43	30	36	46	16	21	24	18	29.2
N_a	1	2	2	2	2	2	2	2	1.8
H_e	0	0.49	0.31	0.46	0.11	0.50	0.08	6.48	0.30
H_o	0	0.50	0.33	0.46	0.13	0.80	0.08	0.56	0.36
Sco 19									
N	43	30	35	45	17	21	23	18	29
N_a	1	5	6	4	2	3	4	3	3.5
H_e	0	0.46	0.41	0.27	0.39	0.09	0.16	0.24	0.25
H_o	0	0.33*	0.43	0.27	0.53	0.09	0.17	0.17	0.25
Sco 23									
N	43	30	35	46	17	21	22	18	29
N_a	1	3	2	1	2	2	2	2	1.8
H_e	0	0.49	0.37	0	0.38	0.52	0.46	0.42	0.33
H_o	0	0.60	0.43	0	0.29	0.52	0.58	0.61	0.39

Note: Populations: HL, Harrison Lake (Red Deer River drainage, Alberta); IR, Ice River (upper Columbia River, B.C.); SK, Skookumchuck Creek (upper Kootenay River, B.C.); RC, Ram Creek (upper Kootenay River, B.C.); CC, Chamberlain Creek (lower Peace River, B.C.); NBR, North Burnt River (lower Peace River, B.C.); UBC, Upper Brazion Creek (lower Peace River, B.C.); BR, Burnt River (lower Peace River, B.C.).

*$P < 0.006$ (Bonferroni-corrected α for eight within-locus simultaneous tests of Hardy–Weinberg equilibrium).

Table 4. Locus, source species, reference, total alleles observed (N_a), sample size (N), molecular size range, and total expected heterozygosity (H_e) for five microsatellite loci assayed in bull trout.

Locus	Source species	Reference	Annealing temperature (°C)	N_a	N	Size range (basepairs)	H_e
Omy 77	Rainbow trout	Morris et al. 1996	56	3	228	279–283	0.45
Sfo 18	Brook trout	Angers et al. 1995	65	2	233	150–156	0.36
Ssa 197	Atlantic salmon	O'Reilly et al. 1996	58	2	225	119–123	0.48
Sco 19[a]	Bull trout	F CTT GAA ATT AGT TAA ACA GC R* CCA AAC TAC CCA ATA ATC	53	11	231	158–216	0.27
Sco 23[a]	Bull trout	F TGT GAA AAG GGG AGA CAG AG 3′ R GGA GAC AGG GGA TGG AAA AG	61	3	236	185–193	0.39

Note: All reactions used the forward primer as the labelled primer unless indicated by an asterisk. All sequences are written 5′–3′.

[a]E.B. Taylor, unpublished data.

trout (i.e., they were homozygous for species-specific alleles at all three nuclear loci), and seven sites contained at least one hybrid individual (i.e., each such individual was heterozygous for at least one locus or was homozygous for alternative species-specific alleles at two or more loci). Two of the zones of sympatry represent confirmations of range extentions for Dolly Varden. Dolly Varden were found in two tributaries of the upper Peace River (Mackenzie River drainage): Thutade Lake (cf. Baxter et al. 1997) and the Omineca River (Fig. 1; Table 5). The extent of hybridization varied among the localities; in samples with at least 15 individuals assayed, the percentage of hybrid-class individuals varied from a high of 25% in the Southgate River to 2% in the Iskut River ($\chi^2 = 16.1$, 4 df, $P = 0.007$) (Table 5).

Phylogenetic groupings within species

Taylor et al. (1999) resolved two phylogenetic groupings of bull trout using restriction site and sequence analysis of five regions of the mtDNA genome. "Coastal" bull trout were found west of the Coastal and Cascade Mountain crests and "interior" bull trout were found east of these mountains. Our current sequence analysis of the ND1 region in Dolly Varden (Appendix, Table A1) also resolved two groupings that differed from one another by about 1.1% in sequence

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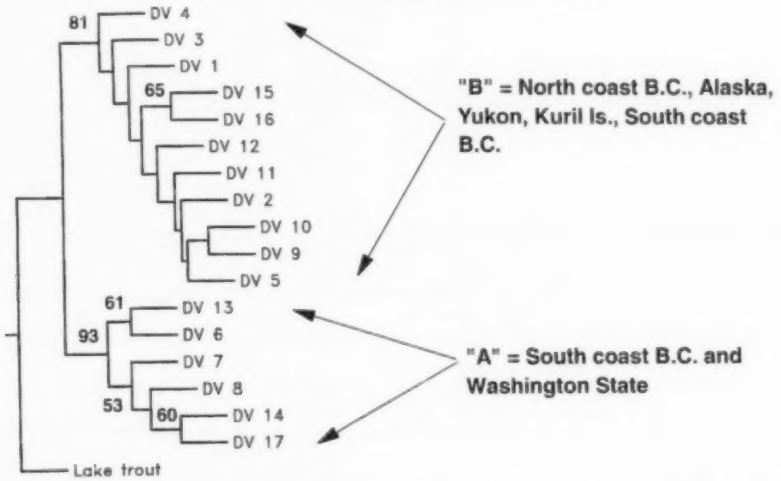
Table 5. Parental species and hybrid identification in selected watersheds.

Sample	Drainage	Dolly Varden	Bull trout	Hybrids
Tulsequah R. (1a)	Taku R.	7		
Nakina R. (1b)	Taku R.		10	
Tatsatua R. (1c)	Taku R.		5	
Chutine R. (2)	Stikine R.	15	1	3
Tahltan R. (3)	Stikine R.			1
Iskut R. (4)	Nass R.	33	13	1
Thutade Lake (5)	Upper Peace R.	49	127	9
Omineca R. (6)	Upper Peace R.	24	2	1
Goathorn Cr. (7)	Skeena R.	15	10	
Toba R. (8)	South coast B.C.	9		1
Southgate R. (9)	South coast B.C.	10	11	7
Squamish R. (10)	South coast B.C.	20	10	

Note: Number codes following river and lake names refer to sites given in Fig. 1.

Fig. 2. Phylogenetic tree of Dolly Varden mtDNA based on parsimony analysis of 503 basepairs of the ND1 gene. Numbers at branch points represent bootstrap percentages based on 1000 resamplings of the sequence matrix. The lake trout ND1 sequence was used as the outgroup. Sample identifications for all sequences are given in the Appendix (Table A2).



(Fig. 2). These two clades of Dolly Varden mtDNA were well supported in the parsimony analysis accompanied by bootstrap analysis (minimum of 81% support). Several restriction sites were diagnostic in the samples that we examined, and we used *Hae* III to resolve the presence of one or both clades in further samples (Table 2; Fig. 3). One clade (A) was found on the Olympic Peninsula, Vancouver Island, and south coastal British Columbia and north to the headwaters of the Nass River. The other clade (B) corresponded to samples from the northwest Pacific Ocean (Kuril Islands), western Alaska, north coastal British Columbia, the Queen Charlotte Islands, and the Peel River (a northwestern Yukon tributary of the Mackenzie River) and extended down to southern Vancouver Island (Figs. 2 and 3).

Combining the 17 Dolly Varden haplotypes with the six bull trout ND1 sequence haplotypes suggested that Dolly Varden mtDNA is paraphyletic; members of all Dolly Varden clade A haplotypes are more closely related to bull trout mtDNA than they are to Dolly Varden clade B haplotypes (61% bootstrap support) (Fig. 4). In fact, bull trout mtDNA is also paraphyetic, as interior bull trout mtDNA clusters separately from a lineage composed of coastal bull trout and group A Dolly Varden mtDNA (63% bootstrap support) (Fig. 4). Enforcing bull trout monophyly and Dolly Varden monophyly resulted in phylogenetic trees that were significantly worse (log likelihood $D = -40.2$, $P < 0.05$) than the maximum-likelihood tree that had the same topology as the parsimony tree (phenogram not shown). The six Dolly Varden ND1 haplotypes that were part of the mtDNA clade with the six bull trout haplotypes, however, were found in individuals that all possessed the rDNA ITS-1, MTB, and GH alleles that are diagnostic for Dolly Varden and vice versa (Redenbach 2000).

Microsatellite polymorphism within populations

In general, microsatellite polymorphism in bull trout across all loci was relatively low (Appendix, Table A2). Most populations had only two alleles at all loci (except for *Sco* 19), and one population (Harrison Lake) was fixed for a single allele at all five loci. There was only one significant departure from Hardy–Weinberg equilibrium involving a reduced number of heterozygotes from expected (*Sco* 19 in Ice

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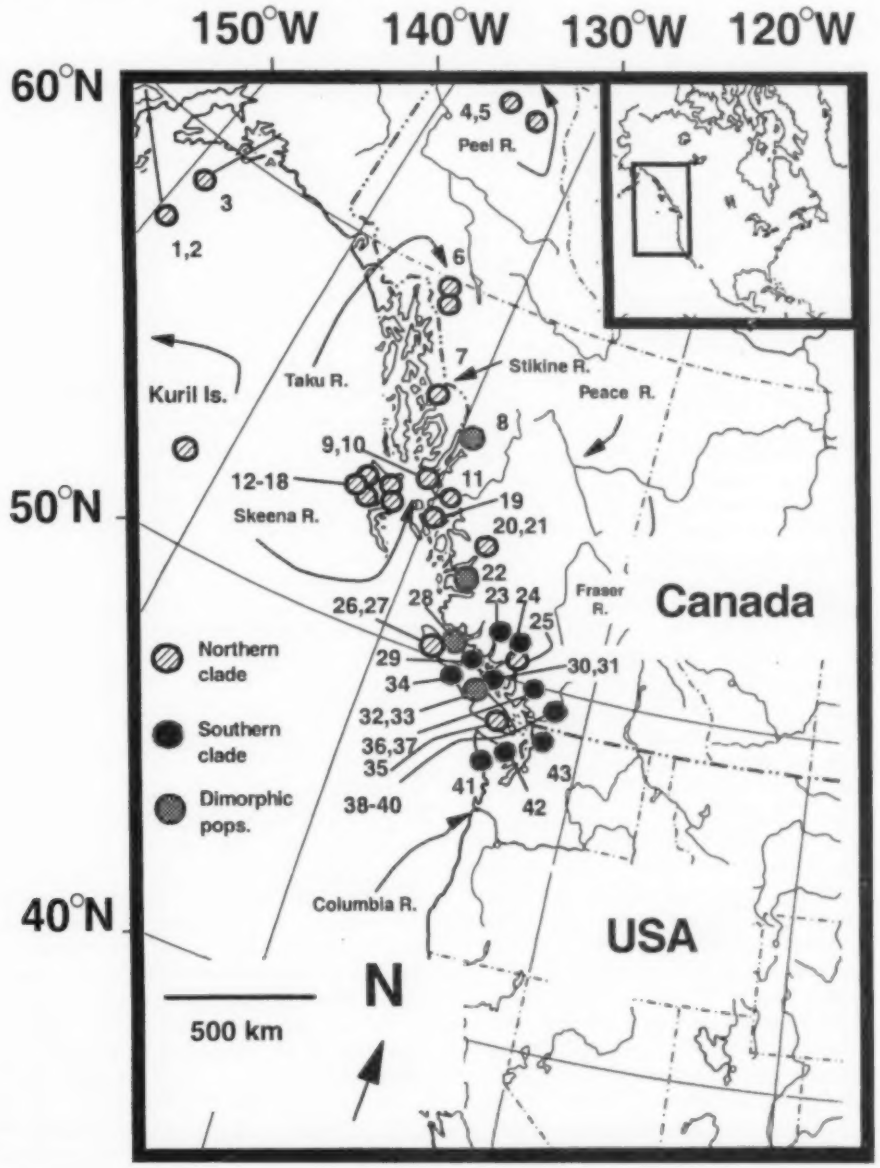
Fig. 3. Geographic distribution of two clades of Dolly Varden mtDNA based on sequence and restriction fragment polymorphism analysis (with *Hae* III) of the ND1 gene. Pies represent the presence of either or both clades but not their relative frequencies. Inset shows sample area in relation to North America. Sample codes are given in Table 2.



River) (Table 3). All loci appeared to be in linkage equilibrium as well (minimum $P = 0.06$ within populations and 0.32 across populations). The populations tended to have low and variable average gene diversities (expected heterozygosities), typically ranging from 0.29 (Ram Creek) to 0.47 (Burnt River). A notable exception was Harrison Lake with an average gene diversity of 0.009 and an observed heterozygosity of 0.0.

Microsatellite polymorphism among populations

There was marked among-population variation in allele frequencies (Appendix, Table A2). Fixation indices (θ) across all eight sample sites ranged from 0.07 (*Sco* 19) to 0.42 (*Sfo* 18 and *Ssa* 197). The overall level of subdivision was high ($\theta = 0.33$, 95% CI = 0.2164–0.3986), which was significantly greater than 0 ($P < 0.005$). Values of θ within watersheds were slightly lower; mean θ within the Columbia/Kootenay and Peace watersheds was 0.143 and 0.189, respectively (both $P < 0.05$).

In pairwise analyses within watersheds, all but one of the population pairs (North Burnt and Burnt rivers, $P > 0.05$) were significantly different from one another in allele fre-

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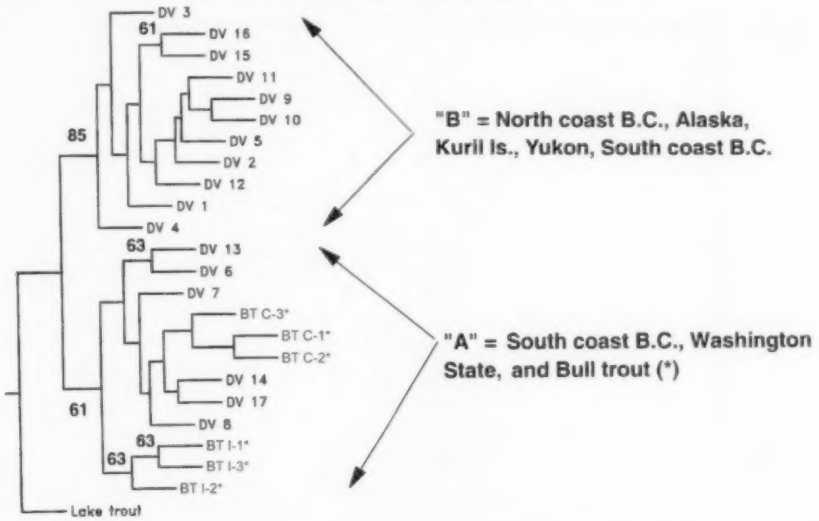
Fig. 4. Phylogenetic tree of Dolly Varden and bull trout mtDNA based on parsimony analysis of 503 basepairs of the ND1 gene. Bull trout sequences (asterisks) are from Taylor et al. (1999). Numbers at branch points represent bootstrap percentages based on 1000 resamplings of the sequence matrix. The lake trout ND1 sequence was used as the outgroup. The annotations "C" and "I" accompanying the bull trout haplotypes designate samples from coastal and interior regions, respectively (see Taylor et al. 1999). Haplotype identities for Dolly Varden are as in Fig. 2 and those for bull trout are given in Taylor et al. (1999).



quencies pooled across loci (maximum $P < 0.001$), but some loci produced more differences than others. Within the upper Kootenay/Columbia system, *Omy* 77 and *Sco* 19 showed all pairwise tests as significant (maximum $P = 0.002$), while in the Peace, *Omy* 77, *Sco* 19, and *Sco* 23 tended to show the greatest number of significant differences (maximum $P = 0.02$). Between watersheds, the greatest differences in allele frequencies were found at *Sfo* 18 and *Sco* 19 (Appendix, Table A2).

Using the theoretical relationship between F_{st} and number of migrants (N_em), $F_{st} \approx 1/(4N_em + 1)$, estimates of exchange among populations within watersheds (Harrison Lake excluded) ranged from two (Ice River and Ram Creek) to three (Skookumchuck Creek and Ram Creek) individuals per generation in the upper Kootenay/Columbia. In the Peace watershed, estimates ranged from one (Upper Brazion Creek and Burnt River) to three (North Burnt and Burnt rivers) individuals per generation.

Hierarchical analysis of microsatellite diversity based on allele frequencies (F_{st}) indicated that most variation resided within populations ($F_{st} = 0.32$) but with significant variation among populations within watersheds ($F_{sc} = 0.28$) and low, but significant variation between the Peace and upper Kootenay/Columbia watersheds ($F_{ct} = 0.06$) (all $P < 0.01$).

Evidence for population bottlenecks

The microsatellite data was subject to the mode-shift indicator for recent population bottlenecks described by Luikart et al. (1998), and three populations showed an excess of medium-frequency alleles suggestive of recent bottlenecks: Skookumchuck Creek, Chamberlain Creek, and North Burnt River. In addition, The Harrison Lake population exhibited a lack of detectable variation across five loci (mean observed heterozygosity of 0.0), suggesting that this population has been subject to strong founder events or population bottlenecks during or following colonization of the lake.

Discussion

Sympatry and hybridization between Dolly Varden and bull trout

Our data confirm and extend suggestions of sympatry and hybridization between Dolly Varden and bull trout that were based on morphological analyses. Cavender (1978) concluded that the species coexisted in the McCloud River (California), Puget Sound, and Skeena and Taku rivers, with two fish identified as hybrids in the upper Skeena River. Haas and McPhail (1991) reported Dolly Varden and bull trout coexisting in the Nass and Stikine rivers as well. Our data confirmed the presence of both species in these watersheds and identified three further areas of sympatry along the southwestern coast of British Columbia (Toba, Southgate, and Squamish rivers). These data, coupled with earlier molecular-based identifications of both species in the upper Peace River (Baxter et al. 1997), the Olympic Peninsula (Leary and Allendorf 1997), and the Skagit River (a tributary of northern Puget Sound; McPhail and Taylor 1995), indicate that Dolly Varden and bull trout come into contact along most of their largely parapatric distributions in northwestern North America.

Bull trout are thought to have originated in the Columbia River basin (Behnke 1980), and dispersal via inland watershed connections occurred throughout the interior of British Columbia and north to the Yukon and east of the Rocky Mountains to the North and South Saskatchewan River systems. Dispersal to the Olympic Peninsula, Puget Sound, and south coastal British Columbia watersheds via the marine environment has also been suggested (Cavender 1978; Behnke 1980; Haas and McPhail 1991). Dolly Varden proba-

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bly colonized most of their southern range via marine dispersal, given their largely coastal distribution and their well-established anadromous behaviour (e.g., DeCicco 1992). Sympatric populations of Dolly Varden and bull trout therefore probably originiated from two processes: double invasions of emerging coastal habitats via marine dispersal and headwater exchanges between inland tributaries of large coastal and interior drainages (e.g., between the Skeena and upper Peace rivers; Baxter et al. 1997). Over their range of contact, of particular curiosity is the absence of bull trout from Vancouver Island (where Dolly Varden are common), which is less than 50 km from sympatric populations on the Olympic Peninsula and the south coast of British Columbia (see Redenbach 2000).

Our data indicate that Dolly Varden and bull trout hybridize over an extensive area of contact. McPhail and Taylor (1995) and Baxter et al. (1997) reported hybridization in two independent watersheds separated by over 1000 km, and therefore, natural hybridization between the two species is clearly a widespread phenomenon. Our surveys were not exhausive in many areas, and sampling was largely opportunistic, but our data suggest that the rate of hybridization is variable among watersheds. For instance, in the Southgate watershed, about 25% of all char examined were classified as hybrids whereas only about 2% were hybrids in the Iskut River sample. Where the ecology of sympatric populations has been studied in detail (McPhail and Taylor 1995; Hagen 2000), size-dependent differences in spawning behaviour and habitat choice appear to play an important role in reproductive isolation between Dolly Varden and bull trout. Perhaps the opportunities for interspecific matings vary among watersheds owing to variability in habitat or life history characteristics of their char populations, and this may contribute to variation in the rate of hybridization (Arnold 1997). An alternative hypothesis is that postmating processes are major determinants of the extent of hybridization and gene flow and that the distinct watersheds that we have sampled vary in the degree to which they select against hybrids.

Intraspecific phylogeny and reticulate evolution in char

Our analysis of ND1 mtDNA sequences in Dolly Varden over its native range demonstrated the presence of two phylogenetic groupings. Taylor et al. (1999) reported similar findings for the same region of mtDNA in bull trout and suggested that the diversification within bull trout was driven by historical isolation and postglacial dispersal from two glacial refugia: the Chehalis and lower Columbia River Valley. Combined analysis of Dolly Varden and bull trout for ND1, however, indicated a a paraphyletic relationship of mtDNA for both species, i.e., some Dolly Varden haplotypes were more closely related to bull trout than to other Dolly Varden and vice versa. More detailed analyses of nuclear ITS-1 and GH nuclear sequences of Dolly Varden and bull trout with paraphyletic ND1 haplotypes indicate that, unlike the mtDNA results, Dolly Varden and bull trout are reciprocally monophyletic (minimum 98% bootstrap support) at these nuclear loci (Redenbach 2000). Our demonstration of current hybridization between the species over a geographic area that includes sites with paraphyletic mtDNA and the clear distinction between the species in terms of nuclear sequences at two loci strongly hint that the presence of two divergent mtDNA clades in Dolly Varden is attributable to introgression of bull trout mtDNA into Dolly Varden. The pattern of mtDNA paraphyly in the presence of nuclear genome distinction is a classic signature of introgression of cytoplasmically inherited genomes documented in a variety of plants and animals (see Arnold (1997) for a review) including char (e.g., Bernatchez et al. 1995). Two observations suggest that that introgression of bull trout mtDNA into Dolly Varden includes ancient hybridization events as well ongoing hybridization. First, the bull-trout-like Dolly Varden mtDNA is found in areas that currently have no bull trout populations (Vancouver Island), and therefore, mtDNA introgression must have occurred in the past (cf. Wilson and Bernatchez 1998; Shimizu and Ueshima 2000). Second, the paraphyletic Dolly Varden and bull trout ND1 sequences are not identical to each other, which suggests that the mtDNA introgression must have occurred long enough ago for sequence polymorphism to acrue within the paraphyletic Dolly Varden sequences.

It is unlikely that either retention of ancestral polymorphism or convergent evolution of sequence variants could explain the paraphyletic relationships between mtDNAs of bull trout and Dolly Varden. First, the shared sequence variants are not distributed throughout the range of both species, the expected pattern for shared ancestral polymorphisms, but appear to be restricted to areas of current sympatry or to areas that are relatively short dispersal distances from sympatric sites. Second, the paraphyletic Dolly Varden and bull trout mtDNAs share six sequence variants, which necessitates six convergent substitutions to explain their similarity. This number of homoplasic events is clearly less parsimonious than similarity through introgression (cf. Bernatchez et al. 1995).

The distinction of Dolly Varden mtDNA into "northern" and "southern" groupings, with the southern grouping probably derived from mtDNA introgression from bull trout, is remarkably similar to variation in cpDNA between several species of plants in northwestern North America. Soltis et al. (1997) described northern and southern clade distinctions in five angiosperms and one fern and suggested that these clades probably stem from isolation in distinct refugia at the southern margins of the Pleistocene ice sheets and further north on the Olympic Peninsula, Queen Charlotte Islands, and (or) islands in the Gulf of Alaska. One herb species, *Tellima grandifora*, however, was unique because the southern group of populations appears to have aquired its chloroplast genome via ancient hybridization and cpDNA capture from a species of *Mitella* (reviewed by Soltis et al. 1997). This cpDNA capture and its possible analogous situation for mtDNA in Dolly Varden may have been facilitated by sympatry of char (and the plants) in southern glacial refugia or by interactions upon secondary contact.

Reciprocal laboratory crosses between Dolly Varden and bull trout apparently show no bias in viability of F1 offspring (Haas and McPhail 1991), which suggests that paraphyly of bull trout and Dolly Varden mtDNAs could result from bidirectional introgression. It appears, however, that a bias in introgression likely exists in the direction of bull trout introgression of mtDNA into the genome of Dolly Varden. First, the geographic distribution of Dolly Varden

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mtDNA clades is not as tightly linked to geography as are those of bull trout (e.g., there is more overlap between clades in Dolly Varden). This suggests that vicariant events may not have been as important in structuring Dolly Varden mtDNA phylogenetic variation and that some other factor may best explain the geographic distribution of mtDNA variants. Second, of the six ND1 sequence sites that diagnose the paraphyletic Dolly Varden and bull trout clade, five are shared with the largely allopatric interior bull trout clade and none are shared with the largely allopatric "northwestern" Dolly Varden clade. Finally, where the two species are currently sympatric, Dolly Varden are typically much smaller at maturity than bull trout (e.g., McPhail and Taylor 1995; Baxter et al. 1997). Baxter et al. (1997) outlined a hypothesis that hybridization is asymmetrical, with smaller-bodied Dolly Varden males acting as "sneakers" during spawning between larger-bodied bull trout male–female pairs. Because mtDNA is, with few exceptions, maternally inherited (Avise 1994), such biased hybridization could provide a mechanism for introgression of bull trout mtDNA into Dolly Varden from backcrossing of F1 hybrids to Dolly Varden.

Population subdivision within bull trout

Bull trout exhibit relatively low levels of within-population variation at microsatellite loci; the number of alleles in each population tended to be low (one to three at all but *Sco* 19), and average heterozygosities were generally less than 50%. In anadromous Arctic char (*Salvelinus alpinus*) from Newfoundland and Labrador, the number of alleles at six loci across seven populations ranged from nine to 48 and expected heterozygosities from 0.19 to 0.97 (Bernatchez et al. 1998). Similarily high heterozygosities were observed at heterologous loci in Arctic char from alpine lakes in Europe (Brunner et al. 1998). Lower heterozygosities were observed in freshwater populations of brook trout (*Salvelinus fontinalis*) in lakes from south-central Quebec (Angers et al. 1995; Angers and Bernatchez 1998), but variability was still generally higher than that observed in bull trout. Low heterozygosity values were also observed in bull trout from the upper Columbia and Klamath rivers at four heterologous loci (P. Spruell, Division of Biological Sciences, University of Montana, Missoula, MT 59812, U.S.A., unpublished data) and in populations sampled from the Lake Pend d'Oreille system (average expected heterozygosities across loci ranged from 0.0 to 0.40; Spruell et al. 1999). These microsatellite data are consistent with earlier studies of allozymes (Leary et al. 1993) and mtDNA (Taylor et al. 1999) that reported relatively low levels of within-population variation at these independent loci. Low heterozygosity values at microsatellite loci in bull trout could result from inherently low mutation rates at these loci for this species, as microsatellites are not necessarily always "hypervariable" (e.g., see Schug et al. 1997). Alternatively, low variability may stem from demographic processes that have reduced effective population sizes in bull trout historically during and following postglacial dispersal (e.g., Hewitt 1996). Reduced population sizes through bottlenecks or founder events would have eliminated considerable allelic variation which has not yet recovered via mutation (cf. Schug et al. 1997).

Notwithstanding low allelic variation at microsatellite loci in bull trout, there is substantial variation among populations in the frequency of alleles that do exist. Average F_{st} values were typically greater than 0.3 and within each region ranged from 0.09 to 0.22. These values are typical of those reported for many other salmonids (e.g., see Wenburg et al. 1998). Our overall value of $F_{st} = 0.33$ is very similar to the value of 0.37 reported by Angers and Bernatchez (1998) for 26 lake populations of brook trout in Quebec. An estimate of effective population size (N_e) can be obtained using the expression $\hat{N}_e \approx H/(1 - H)/4\mu$, where H is heterozygosity and μ is the mutation rate (see Waples 1998). We used the average expected heterozygosity across loci for all populations (0.33) excluding the Harrison Lake population, which was monomorphic at all loci, and a mutation rate of 10^{-4}, which is typical for many microsatellite loci (e.g., reviewed in Feldman et al. 1999). This expression yields an estimated N_e of about 1200 fish among the tributaries that we sampled within each of the Columbia/Kootenay and Pine watersheds. Our estimates of migration among populations under an island model of population structure were typically less than five individuals within each of the Pine and Kootenay watersheds. These data, although subject to a number of assumptions (see Waples 1998; Whitlock and McCauley 1999), strongly suggest that bull trout populations are highly isolated from each other demographically and genetically (cf. Spruell et al. 1999).

Our data suggest that genetic subdivision was greater within the Peace region than in the Columbia/Kootenay, which was surprising given the greater geographic distance among the latter samples. Greater differentiation in the Peace may stem from the inclusion of samples from above impassable (apparently) waterfalls in Chamberlain Creek and Upper Brazion Creek. By contrast, at least between Ram Creek and Skookumchuck Creek on the Kootenay River, fewer habitat structure induced impediments to bidirectional gene flow appear to exist. This hypothesis is being tested by assaying more populations while also characterizing sample sites in terms of geomorphological features that might aid in understanding what landscape features might organize variation within and between populations of bull trout (cf. Angers et al. 1999).

Conservation implications

Our analyses demonstrate the widespread sympatry of Dolly Varden and bull trout over much of their largely parapatric geographic ranges. The two species hybridize in areas of contact and some gene flow (introgression) occurs between them (Baxter et al. 1997; Redenbach 2000) and presumably has occurred at least since postglacial times (i.e., over the last 10 000 – 15 000 years). Despite such gene flow, Dolly Varden and bull trout are clearly distinct gene pools and diagnosable over their ranges and, in particular, in areas of sympatry. Distinction in sympatry and in the face of gene flow is the "acid test" of the biological species concept (Avise 1994), and consequently, the recognition of Dolly Varden and bull trout as distinct species is clearly warranted. Nevertheless, it is also clear that the two species' evolutionary histories have not been completely independent. Rather, our phylogeographic analyses suggest that their evolutionary genetic interactions have been frequent and continue to the present day. It is possible that such interactions may have

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contributed novel genotypes to both species and to their respective evolutionary trajectories and that such processes may act as creative forces in the current biodiversity of both taxa (Arnold 1997). Our analyses do caution, however, that similar patterns of mtDNA diversification (e.g., two divergent lineages within both Dolly Varden and bull trout) can stem from different causes (introgression versus vicariance, respectively). Different evolutionary causes of lineage diversification within species deserve recognition, particularly with respect to mtDNA, which is often used as a basis for designation of conservation units below the species level (e.g., Moritz 1994).

On a much finer scale of geographic variation, microsatellite variation in bull trout demonstrates that significant amounts of genetic subdivision exist among populations within relatively small areas (e.g., within the Pine River drainage) as well as across larger areas (between the Peace and Kootenay drainages) within a single phylogenetic lineage (i.e., the interior bull trout clade of Taylor et al. 1999). These data imply a high degree of demographic independence among populations within watersheds, an inference that should be considered during management plans for these watersheds. For instance, our data suggest that populations undergoing large reductions in population size will not be "rescued" from extinction by migration from other populations, at least in the short term (also see Spruell et al. 1999). Further, the low levels of molecular variation in bull trout (also seen in other areas for microsatellites, in mtDNA, and allozymes) strongly hint that bull trout have been subjected to large and repeated reductions in effective population size. This may reflect their relatively recent postglacial dispersal into the upper Columbia/Kootenay and Peace River watersheds from refugia outside British Columbia (see Taylor et al. 1999), with bottlenecks and founder events during colonization reducing variation (Hewitt 1996). Reduced variation, in addition to the evidence for recent bottlenecks from our analysis, may indicate that bull trout are vulnerable to potentially deleterious effects of reduced population size, i.e., the molecular data suggest periodic reductives in population sizes that may foster inbreeding (Saccheri et al. 1998). Consequently, bull trout may be a species that is especially sensitive to human-induced reductions in population size that exceed the apparently natural historical reductions inferred from our data.

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Appendix

Table A1. Polymorphic sites in 503 basepairs of aligned ND1 sequences for char sampled in the study.

					11	11	11	222	22	23	33	33	34	44	45	55
	11	22	24	68	14	45	56	113	78	90	00	15	54	56	70	00
	759	23	55	60	01	31	68	261	27	10	35	44	91	03	10	13
DV 1	TGG	TT	GT	CA	CT	GG	CT	ACT	CT	CA	TC	AG	CG	AT	TG	GC
DV 2	.C.	G.	..	..	..	..	..	...	..	..	..	..	..	..	..	..
DV 3	...	..	CC	..	..	..	..	...	..	..	..	..	..	..	..	..
DV 4	...	..	..	..	..	..	..	...	T.	..	..	..	..	..	..	..
DV 5	.C.	..	T.	.G	..	..	..	...	..	..	.T	..	T.	..	..	..
DV 6	...	..	A.	..	..	..	..	...	..	..	.T	G.	.A	..	CA	.T
DV 7	...	G.	..	..	..	..	..	...	..	..	.T	G.	.A	..	CA	.T
DV 8	...	..	..	..	..	..	..	...	..	..	..	..	.A	..	CA	.T
DV 9	.C.	..	A.	..	..	..	..	...	..	..	..	.A	..	..	..	..
DV 10	.C.	..	A.	T.	..	..	..	...	..	..	..	..	..	..	..	..
DV 11	CC.	..	..	..	..	..	..	...	..	..	..	.A	..	..	..	..
DV 12	...	..	..	T.	..	..	..	...	..	..	.T	G.	..	..	..	..
DV 13	...	..	A.	..	..	..	T.	...	..	..	.T	G.	TA	..	CA	.T
DV 14	...	..	..	..	..	..	..	...	..	..	..	..	.A	..	CA	.T
DV 15	..A	..	..	..	..	..	..	...	..	..	C.	..	..	..	..	..
DV 16	..A	.A	..	..	..	..	..	...	..	..	.T	G.	..	..	..	..
DV 17	...	..	..	..	..	..	..	...	..	..	.T	G.	TA	.C	CA	.T
BT C-1	...	..	..	T.	..	..	..	...	..	..	.T	G.	.A	G.	CA	.T
BT C-2	...	..	..	T.	..	..	..	...	..	..	.T	G.	.A	G.	CA	.T
BT C-3	...	..	..	..	..	A.	..	...	..	..	.T	G.	TA	..	CA	.T
BT I-1	...	..	..	..	..	..	.C	...	..	T.	.T	G.	.A	..	.A	.T
BT I-2	...	..	..	..	..	..	..	...	..	T.	.T	G.	.A	..	.A	.T
BT I-3	...	..	..	..	..	T.	.C	...	..	T.	.T	G.	.A	..	.A	TT
Lake Trout	.	.	.	.	T	C	.	.	G	T	C	.	C	.	G	.	T	G	.	.	.	.	.	A	.	.

Note: The region sequenced includes a portion of the 16S rRNA gene (basepairs 1–199), Leu-tRNA (basepairs 200–275), and the NADH-1 gene (basepairs 276–503). Haplotype names refer to those in Fig. 2.

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Table A2. Allele frequencies at five microsatellite loci in eight bull trout populations.

	Locus and alleles (basepairs)																	
	Omy 77				*Sfo* 18			*Ssa* 197			*Sco* 19				*Sco* 23			
Population	*N*	279	283	287	*N*	150	156	*N*	119	123	*N*	174	200	RA	*N*	185	191	193
HL	43	0.000	1.000	0.000	43	1.000	0.000	43	0.000	1.000	42	0.000	1.000	0.000	43	0.000	1.000	0.000
IR	30	0.300	0.700	0.000	30	1.000	0.000	30	0.483	0.517	30	0.000	0.717	0.283	30	0.033	0.350	0.617
SC	36	0.000	0.681	0.319	36	0.847	0.153	36	0.806	0.194	35	0.100	0.757	0.143	35	0.000	0.243	0.757
RC	45	0.000	0.422	0.578	45	0.944	0.056	46	0.641	0.359	45	0.000	0.844	0.156	46	0.000	0.000	1.000
CC	16	0.000	1.000	0.000	17	0.500	0.500	16	0.063	0.938	17	0.265	0.735	0.000	17	0.000	0.265	0.735
NBR	21	0.000	0.643	0.357	21	0.500	0.500	21	0.500	0.500	21	0.024	0.952	0.024	21	0.000	0.519	0.481
UBC	22	0.000	0.273	0.727	25	0.280	0.720	24	0.042	0.958	22	0.022	0.913	0.065	26	0.000	0.519	0.481
BR	15	0.000	0.900	0.100	16	0.406	0.594	15	0.389	0.611	36	0.056	0.861	0.083	18	0.000	0.694	0.306

Note: Sample sites: HL, Harrison Lake; IR, Ice River; SC, Skookumchuck Creek; RC, Ram Creek; CC, Chamberlain Creek; NBR, North Burnt River; UBC, Upper Brazion Creek; BR, Burnt River. To conserve space, nine alleles at *Sco* 19 (158, 190, 192, 198, 202, 204, 206, 210 and 216 basepairs) have been pooled under the designation "RA" for rare alleles.

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Forest harvest impacts on water quality and aquatic biota on the Boreal Plain: introduction to the TROLS lake program

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Abstract: Eleven headwater lakes in Alberta's Boreal Plain were monitored for nutrients and plankton 2 years before and 2 years after variable watershed harvesting (harvesting mean 15%, range 0–35%). After harvesting, variations in annual precipitation resulted in lake water residence times that differed by an order of magnitude from one year to the next. During the first posttreatment year, total phosphorus concentrations increased (overall 40%) in most lakes; however, response was most consistent in lakes that were shallow and the water column mixed or weakly thermally stratified. Chlorophyll *a*, cyanobacteria (*Aphanizomenon–Anabaena*), and cyanotoxins (microcystin-LR) increased after harvesting, primarily in shallow lakes. Zooplankton abundance and biomass decreased after harvesting, particularly in stratified lakes where edible phytoplankton biomass declined. In the weakly or nonstratified lakes, declines in zooplankton biomass were associated with higher cyanobacterial biomass and cyanotoxins. Posttreatment change in total phosphorus concentration was strongly related to weather (greatest response in a wet year) and relative drainage basin size (drainage basin area to lake volume, $r^2 = 0.78$, $P << 0.01$). There was no evidence that buffer strip width (20, 100, and 200 m) influenced lake response. These results suggest that activities within the entire watershed should be the focus of catchment–lake interactions.

Résumé : Nous avons fait le suivi des nutriments et du plancton dans onze lacs situés en tête de bassin hydrographique dans la Plaine boréale de l'Alberta 2 ans avant et 2 ans après des récoltes forestières variables (en moyenne 15% de la surface du bassin hydrographique, étendue de 0 à 35%). Après la récolte, les changements dans les précipitations annuelles ont entraîné des variations dans la durée du séjour de l'eau dans les lacs, par un facteur de dix, d'une année à une autre. Durant la première année après la récolte, les concentrations totales de phosphore ont augmenté (40% en tout) dans la plupart des lacs; cet effet a cependant été plus uniforme dans les lacs peu profonds où le brassage de la colonne d'eau était complet ou chez lesquels la stratification thermique était faible. Les concentrations de chlorophylle *a* et de cyanotoxines (microcystin-LR) et les densités de cyanobactéries (*Aphanisomenon-Anabaena*) ont crû après la récolte, particulièrement dans les lacs peu profonds. En revanche, l'abondance et la biomasse du zooplancton ont décliné après la récolte, surtout dans les lacs stratifiés où la biomasse de phytoplancton comestible avait diminué. Dans les lacs peu ou pas stratifiés, le déclin de la biomasse du zooplancton était associé à une augmentation de la biomasse des cyanobactéries et de la concentration des cyanotoxines. Les changements dans les concentrations totales de phosphore après la récolte étaient fortement reliés aux conditions météorologiques (avec les effets les plus marqués au cours de l'année pluvieuse) et à la taille relative du bassin hydrographique (surface du bassin de drainage par rapport au volume du lac, $r^2 = 0,78$, $P << 0,01$). Il n'y a pas de preuve que la largeur (20, 100 et 200 m) de la bande verte de protection ceinturant le lac ait changé les effets dans le lac. Ces résultats indiquent qu'il est nécessaire de tenir compte de toutes les activités qui se passent dans l'ensemble du bassin hydrographique pour étudier les interactions entre le lac et son bassin.

[Traduit par la Rédaction]

Introduction

Water quality in lakes is a function of both internal and external processes. Disturbances in a watershed (e.g., agriculture, deforestation) can alter external nutrient inputs and runoff (Cooke and Prepas 1998; Lamontagne et al. 2000), thereby changing water quality. Internal recycling of added nutrients through biogeochemical processes (Riley and Prepas 1984) can be a concern, particularly in lakes within sedimentary basins (Shaw and Prepas 1989).

Postharvest changes in nutrient concentrations have been documented in streams (Holopainen and Huttunen 1998) and

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occasionally in lakes (Rask et al. 1998). Impacts at many trophic levels (primary producers to vertebrates) have been attributed to these changes (Rask et al. 1998). Buffer strips, unharvested areas left around lakes and streams, may be effective in moderating water quality changes in disturbed watersheds through retention of runoff and water-soluble nutrients (Ahtiainen 1992). Some information exists on the effectiveness of buffer strips on the Canadian Boreal Shield and west coast of Canada (Newbold et al. 1980); however, there is no information on the Boreal Plain. Given differences in forest composition, till depth, precipitation patterns, and forestry practices, there is little possibility to extrapolate from studies in other regions to the Boreal Plain of western Canada.

The Boreal Forest comprises 82% of Canada's forests. This study is set in the mixedwood ecoregion of the Boreal Plain, located primarily in the province of Alberta. This semiarid region is characterized by deep loamy tills and has both upland and lowland habitat. Since 1992, forest harvesting has increased in northern Alberta, with over 75% of the mixedwood forest leased to forest companies. Lake water on the Boreal Plain is similar to that in other sedimentary basins, being relatively high in total phosphorus (TP) and total dissolved P (TDP) concentrations, while nitrogen (N), particularly inorganic N (IN), is not necessarily correspondingly high (Mitchell and Prepas 1990). Lake water in this region is also relatively low in ions such as iron and calcium, which can bind with P and reduce internal P cycling (Manning et al. 1994). Relatively small disturbance in the watershed may be sufficient to disrupt the P-binding potential of the bottom sediments (Manning et al. 1999), thereby increasing the chance of internal P release, and subsequent eutrophication, in lakes on the Boreal Plain.

Lakes on the Boreal Plain have algal communities characterized by a relatively high proportion of cyanobacteria (Trimbee and Prepas 1987). Relative or absolute changes in available nutrient concentrations, particularly as they impact on P and (or) N, may create conditions conducive to further proliferation of cyanobacteria (Kotak et al. 1995). Cyanobacteria and their associated toxins may impact aquatic food webs (Ghadouani et al. 1998). In oligomesotrophic lakes of the Boreal Forest in eastern Canada, recent studies also indicate that harvesting may impact planktonic communities through changes in nutrients and organic matter in lake water (Patoine et al. 2000; Planas et al. 2000). Primary productivity and biological oxygen demand increase together, often resulting in habitat restriction and (or) loss of oxygen-sensitive fauna (Dinsmore et al. 1999). The Terrestrial and Riparian Organisms, Lakes, and Streams (TROLS) program was created to evaluate the effects of forest harvesting and buffer strips on terrestrial and riparian organisms, lakes, and streams. This paper serves as an introduction to the 12 TROLS lakes and their biotic communities and watersheds.

In this paper, we focus on annual trends and examine the hypotheses that (*i*) changes in TP and IN concentrations in lakes on the Boreal Plain can be predicted from a combination of external and internal features, (*ii*) increased TP concentration will be mirrored in chlorophyll *a* (Chl *a*) concentrations, and a proportional increase in cyanobacteria and associated cyanotoxins, and (*iii*) zooplankton will respond to bottom-up changes as a function of changes in edible algae and cyanobacteria biomass and top-down planktivory.

Materials and methods

TROLS program and study area

The study lakes are located in the southern portion of Alberta's Boreal Mixedwood Ecoregion (Table 1). This region is characterized by sedimentary bedrock overlaid by upland moraines and lacustrine deposits from the last glaciation. Upland mesic stands are characterized by both mixed and pure stands of trembling aspen (*Populus tremuloides*) and white spruce (*Picea glauca*). Black spruce (*Picea mariana*), balsam poplar (*Populus balsamifera*), paper birch (*Betula papyrifera*), and tamarack (*Larix laricina*) dominate wetter sites.

Selection criteria for the lakes included (*i*) in or near townships slated for harvesting between 1995 and 1998 by Alberta Pacific Forest Industries Inc., (*ii*) >50% of their circumference treed by mature (>50 years) aspen with no recent (50 years) watershed disturbance, (*iii*) headwater, (*iv*) surface areas <200 ha, and (*v*) maximum depth >2 m. All lakes were inaccessible by road. Due to the limited number of lakes to choose from and logistic necessity, one of the 12 lakes, SCL200, was downstream of two other study lakes (SCL100, SCL800). SCL200 functioned more as a widening of a river, with average water residence ≥17-fold shorter than that of the headwater lakes in this study (0.1 versus ≥1.8 years) (Table 1). Although basic data are presented to provide a baseline for all TROLS lakes, SCL200 diverged sufficiently from the other study lakes that unless otherwise stated, it will be introduced but not included in analyses.

The 12 TROLS lakes are clustered in three regional blocks of four lakes each: South Pelican Hills (SPH), South Calling Lake (SCL), and Lac La Biche (LLB). Each block had a lake designed as reference (no cutting within 800 m, the maximum width feasible given timber requirements) or 20-, 100-, or 200-m buffer strip. Following lake selection, accidental harvesting occurred in one of the reference drainage basins (LLB800, February 1994, 9% within the drainage basin) (Table 1). Most forest harvesting was conducted between October 1996 and February 1997. A few trees were removed around SCL20 to connect cutblocks in February 1998. Although the original plan was to result in 30% harvesting within 800 m of the treatment lakes, the overall percent cut in the targeted watersheds was 17% within 800 m (Table 1). In 1997, the economic climate precluded correcting the discrepancies.

Sampling and analysis

Each year, the deepwater spot was marked in each lake and designated the main site. From May to September of 1995–1998, on a continuous rotation, each lake was visited on average twice per month, and, unless otherwise noted, measurements were made at the main site. Measurements at this site included Secchi depth, water level, light penetration (LI-COR model LI-1000 datalogger), water temperature, and dissolved oxygen (DO) (YSI model 59 DO/Temp meter at 1-m intervals from lake surface to within 1 m of the lake bottom). In each lake and on each sampling date, water samples for chemical, Chl *a*, and phytoplankton analyses were collected from the euphotic zone at several locations and then pooled and subsampled. The euphotic zone, defined as from the lake surface to the depth at 1% light penetration, was sampled with weighted Tygon™ tubing fitted with a one-way foot valve.

Water samples for TP and TDP were analyzed with the modified potassium persulphate technique (Kotak et al. 1995). Total dissolved N (TDN) samples were photocombusted within 48 h in an ultraviolet digester and analyzed. Ammonium (NH_4) and nitrite plus nitrate ($NO_2 + NO_3$) samples were analyzed with a Technicon

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Table 1. Location, vegetation, and physical features of the TROLS lakes and watersheds.

						% vegetation composition						% cut						
Lake	Thermal regime	DBA/*V* (m^{-1})	Latitude	Longitude	Watershed slope (%)	Decid	Con	Wet[a]	Rich fens	Swamps and marshes	DBA (ha)	DBA	800-m buffer	A_0 (ha)	Z (m)	Volume ($\times 10^4$ m^3)	Z_{max} (m)	WRT (years)
SPH20	S	1.4	55.4200	113.7050	4.5	45	42	13	6	7	1053	28	33	157	4.7	736	9.4	>16
LLB800	S	1.4	55.2000	111.6350	3.6	84	3	14	<1	10	455	9[b]	6[b]	105	3.5	363	8.7	>17
SCL20	S	2.6	55.1875	113.6550	3.5	81	6	11	3	7	678	3.0[c]	10[c]	52	4.8	250	12	6.3
LLB100	S	2.8	55.1050	111.7575	4.7	85	9	5	0	5	109	35	35	14	2.7	37.7	6.0	5.4
SCL100	S	4.0	55.1300	111.6567	3.0	70	3	25	15	9	292	10	16	18	3.5	63.6	7.6	3.8
SPH200	S	5.8	55.1700	111.9050	3.7	59	29	13	8	3	964	22	21	44	3.7	164	9.1	2.0
LLB20	N	2.6	55.0850	113.7775	8.0	83	7	10	4	4	612	26	29	62	3.5	219	5.8	4.3
LLB200	N	3.4	55.1233	113.7250	3.3	73	4	22	9	12	189	12	12	33	1.6	54.7	4.7	7.6
SCL800	N	4.6	55.3750	113.6300	1.1	82	1	16	6	9	742	0	0	75	2.1	156	3.0	4.6
SPH800	N	4.2	55.3875	113.6325	3.9	59	15	21	21	1	650	6	0	65	2.3	150	3.9	3.7
SPH100	N	10.3	55.3550	113.7250	3.9	60	19	22	10	12	840	10	30	56	1.4	76.2	2.6	1.8
SCL200[d]	N	82	55.1050	113.7342	4.0	70	5	17	5	11	5669	9	14	109	0.6	69.1	2.1	0.12

Note: Lakes are organized as two groups based on thermal regime and within each group on the basis of the ratio of drainage basin area to lake volume (DBA/*V*). The percentage of the 800-m buffer cut refers to the total area cut within the drainage basin within 800 m of the lake. Values for lake features are means for 1995–1998. S, stratified; N, weakly or nonstratified; Decid, deciduous; Con, conifer; Wet, wetland; A_0, surface area; Z, mean depth; Z_{max}, maximum depth; WRT, water residence time.

[a]Wetlands are composed of bogs, rich fens, and swamps and marshes. The study area had no poor fens. The remainder of the area consists of other small lakes.

[b]Harvesting within the LLB800 drainage basin and 800-m buffer area occurred in February 1994.

[c]In 1996–1997, 3.6% of the SCL20 drainage basin was harvested. In 1997 and 1998, an additional 0.4% was harvested (estimated).

[d]SCL200 is not a headwater lake.

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autoanalyzer (Stainton et al. 1977). Particulate N was measured on known volumes of sample filtered through GF/F filters and combusted in a CHN analyzer. Samples for dissolved organic carbon (DOC) and cation analyses were acidified (pH < 2); for DOC, sparged subsamples were run on an Ionics Corporation 1505 programmable C analyzer with a platinum catalyst for cations on a Perkin Elmer 3300 atomic absorption spectrophotometer. Sulphate (SO_4) and chloride (Cl) analyses were run on a Dionex 2000I/SP ion chromatograph fitted with an AS4A-Sc high-capacity anion exchange column. Silica (Si) concentrations were determined spectrophotomically (Environment Canada 1979). pH was measured in the field with a Fisher Scientific Accumet 1001 meter. Alkalinity (HCO_3, CO_3) was determined on a Mettler DL autotitrator. Colour of raw water was measured at 440 nm with a Milton Roy 1001 spectrophotometer (Cuthbert and del Giorgio 1992) and conductivity with a Radiometer CDM 83 meter.

Lakes were divided into two categories, stratified ($Z_{max} \geq 6$ m) and weakly or nonstratified or shallow (for headwater, $Z_{max} < 6$ m), based on thermal profiles from June through August (i.e., summer) over this 4-year study. The water column of the former was thermally stratified (i.e., temperature changed ≥1°C at some depth changes of 1 m) for most (≥75%) observations, while for the latter, the water column was mixed most sampling days. Within the two categories and following patterns in lake nutrient concentrations in undisturbed lakes on the Boreal Plain (Prepas et al. 2001), data presentations were organized from largest to smallest ratio of drainage basin area to lake volume or relative drainage basin area (DBA/*V*).

Dramatic increases (Δ) in euphotic zone TP were assigned to internal sources when water column temperature and DO profiles indicated recent mixing of previously stratified waters and to external sources when monthly and daily regional runoff or rainfall (nearest station) indicated substantive surface input. To determine baseline TP values for each study year, we used average euphotic zone TP concentrations excluding dates with major internal or external events (i.e., mixing or rainfall). Once the measured ΔTP had been assigned to one of the two P sources, baseline TP was subtracted from the average summer TP concentration for that lake and the resulting difference was proportioned into external and internal fractions.

DBAs were estimated from 1 : 50 000 topographic maps and 1 : 15 000 aerial photographs. In cases where delineations were questionable, areas were verified by ground crews. Major vegetation types (bogs, fens, swamps, marshes, coniferous and deciduous tree cover) within each watershed were determined from 1 : 15 000 aerial photographs as per the Alberta wetland inventory standards (Halsey and Vitt 1997). Watershed slope for each lake was calculated as the average rise/run of 10–15 transects around each lake. Bathymetry of each lake was determined from four to six transects with five to seven nodes each.

The annual water residence time (WRT) for each lake was calculated by

$$\mathrm{WRT} = V/((P + R - E_{pot})/1000A_0)$$

where V represents lake volume (cubic metres), A_0 represents surface area (square metres), and P, R, and E_{pot} represent precipitation, runoff, and potential evaporation (millimetres per year), respectively. Annual lake volumes were calculated with the average lake depth for that year. Surface areas were interpolated from water depth and lake basin slope obtained from bathymetric maps. Potential evaporation was estimated with Thornthwaite's equation (Gray 1970). Annual precipitation and average air temperatures were obtained from Environment Canada climate stations at the nearby towns of Athabasca, Slave Lake, Cold Lake, and City of Fort McMurray. Precipitation and temperature averages for each of the three TROLS regions were determined for a central point, with data from the four weather stations weighted inversely for distance from the central point. Unit area runoff for the three regions was estimated from average annual discharge of two (SPH, SCL) or three (LLB) nearby streams gauged by the Water Survey of Canada. To calculate annual runoff for each gauged stream, total annual discharge was divided by drainage area. To calculate average annual WRT for each lake, an upper bound of 50 years was imposed on estimated annual WRT.

Phytoplankton samples were stored in brown glass bottles and preserved in Lugol's solution until analysis. Phytoplankton species were identified, measured, and counted with a Leica DM-1RB inverted microscope, as described in Planas et al. (2000). Algal biomass of dominant taxa was expressed as biovolume. Samples for Chl *a* analyses were filtered through a 20-µm Nitex™ mesh, and thus, we could separate edible (EP: <2–20 µm) and nonedible (NEP: >20 µm) fractions calculated from total Chl *a* (TChl*a*) – EP. Phytoplankton from both fractions was concentrated onto Whatman GF/C filters at –50 kPa and analyzed by Ostrofsky's ethanol extraction technique (Bergmann and Peters 1980). Samples for microcystin-LR (MCLR) analyses were collected from a depth of 2 m to the surface at the main site (Kotak et al. 1995). Subsequently, samples were lyophilized, homogenized, sonicated, filtered, evaporated, and run on a reverse-phase high-performance liquid chromatograph (Waters LC module 1 HPLC with a 15-cm NovaPak C-18 reverse-phase column) following Zurawell et al. (1999).

Triplicate zooplankton samples were collected in the euphotic zone of each lake by vertical hauls with a 53-µm mesh size cantilevering net (Filion et al. 1993). Zooplankton was narcotized in the field with carbonated water and preserved in 4% buffered formaldehyde solution. Each sample was made up to a uniform volume (60 or 120 mL) depending on the initial volume of concentrated zooplankton samples. An aliquot (20% of the volume) was then taken from each sample date (six to seven) and pooled to give a composite seasonal (May–September) sample for each replicate; all three replicates were then pooled. This coarse approach provided a general picture of variation in the zooplankton community among lakes and years. Species were identified on subsamples of 1.5 mL for rotifers and 3 mL for crustaceans (Pinel-Alloul 1993). Immature stages of calanoids were distinguished from cyclopoids. To evaluate the biomass of each species, we measured the size of ≤240 specimens of each crustacean (length) and rotifer (length and width) species and converted these to dry weight based on length–weight relationships (Malley et al. 1989). Species abundance and biomass were summed to estimate abundance (individuals per litre) or biomass (milligrams per cubic metre) of each taxonomic group (Rotifera, Cladocera, Calanoida, Cyclopoida) and of the total community.

Fish were collected via overnight sets of multimesh benthic gill nets (42 × 1.5 m, with fourteen 3-m-wide panels of 6.25- to 75-mm bar mesh) during year 1, year 2, and year 4, with total effort adjusted for lake size (area, depth). Catch-per-unit-effort (CPUE) was determined as fish per net per hour (W. Tonn and A. Danylchuk, University of Alberta, Edmonton, Alta., unpublished data).

Unless otherwise noted, data were analyzed following Sokal and Rohlf (1980). Data were tested for normality with Kolmogorov–Smironov analysis; nonnormal variables were transformed with a $\log_{10}$ transformation. Because thermal stratification is a strong determinant of lake biological response to nutrients (Riley and Prepas 1984), we divided the lakes into the stratified and weakly or nonstratified categories for the bulk of our analyses. For the zooplankton data, we used nonparametric Kruskal–Wallis one-way analysis of variance (ANOVA) by ranks and Wilcoxon signed-ranks tests to evaluate pre- and post-harvest changes. Unless otherwise noted, for the other water quality and phytoplankton data, comparisons of pre- and post-harvest changes between lake types were performed with Fisher tests (χ^2) for combining probabilities from independent tests of significance. Multiple linear regression and Pearson

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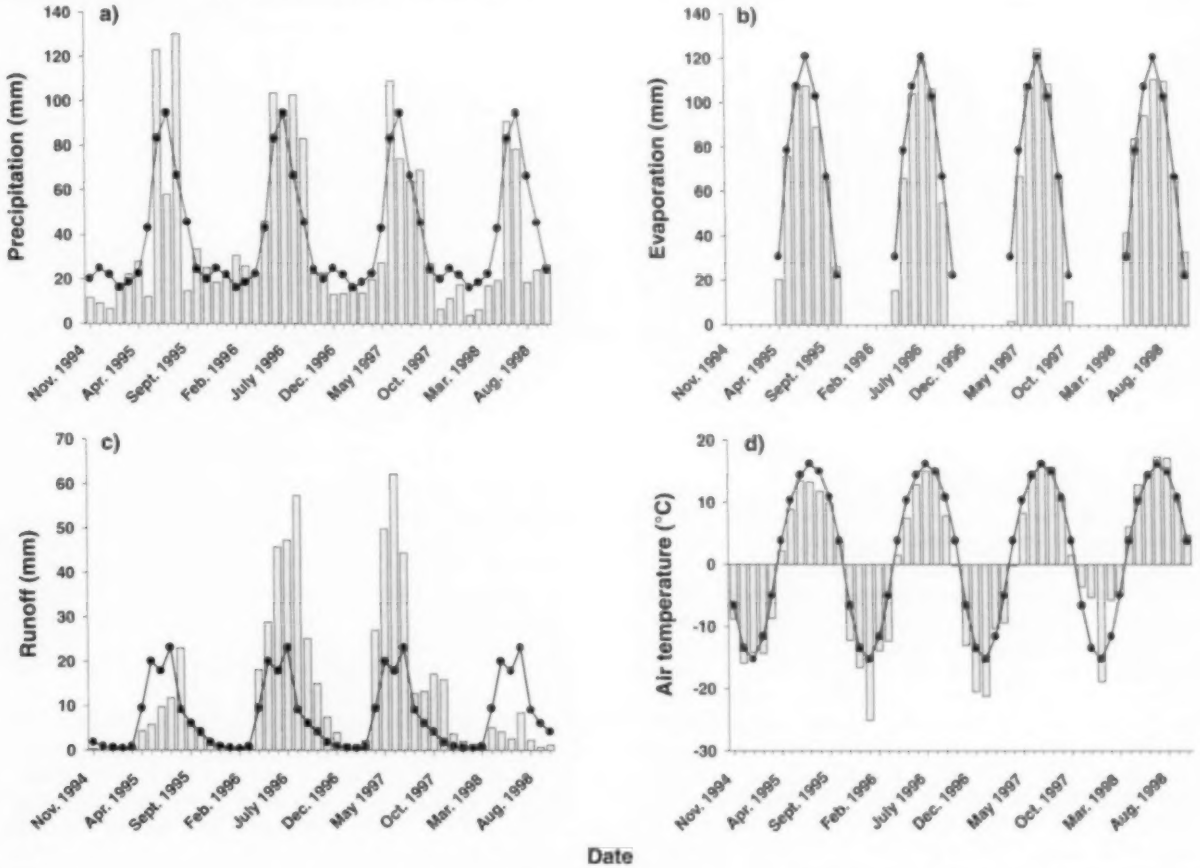
Fig. 1. Monthly (*a*) precipitation, (*b*) evaporation, (*c*) runoff, and (*d*) air temperature for the TROLS region. Bars are monthly means and circles represent long-term means between 1970 and 1994.



correlation analyses were used to evaluate the influence of environmental factors and watershed disturbance on nutrient concentrations and plankton communities; the strongest independent variables were selected based on forward or backward stepwise variable selection methods. Multilinear regressions were performed as colinearity verifications with Durbin–Watson and variance inflation factor indices. The normality of the model residual distribution (comparing observed and predicted values) was tested with the Shapiro–Wilkes *W* test. All the correlation analyses were based on summer averages, and the Bonferroni correction for multiple testing was applied (Legendre and Legendre 1998). A power analysis on pretreatment TP indicated that median differences of 17 $\mu g \cdot L^{-1}$ were required to detect posttreatment differences 90% of the time. Differences were considered significant at $P < 0.05$.

Results and discussion

Hydrology, lake morphometry, and chemistry

The TROLS lakes are situated in an endorheic region in which annual long-term (1970–1994) lake evaporation exceeds precipitation by 11% (529 and 477 mm·year^{-1}, respectively) and runoff is low (25–208 mm·year^{-1}). Evaporation is relatively constant from year to year, and small changes in precipitation have a large influence on runoff (Fig. 1), which during this study varied over 10-fold (27–274 mm·year^{-1}). The study consisted of two dispersed relatively dry years (1995 and 1998, up to 33% less than the 25-year mean precipitation), two consecutive wet years (1996 and 1997, up to 28% more than the 25-year mean), and two consecutive 2-year periods with summer air temperatures less than (1995 and 1996) and greater than (1997 and 1998) the long-term average (Fig. 1). The study area has low relief (average watershed slope 1.1–8%) and the lakes are relatively shallow (mean depth <5 m) (Table 1). The drainage to lake basin area ratios (DBA/A_0) of the headwater lakes varied from 4 to 22, typical of headwater lakes in the boreal forest (Lamontagne et al. 2000; Prepas et al. 2001). Average 4-year WRT for these lakes varied from 1.8 to >17 years (Table 1), while estimates for individual years in individual lakes ranged from 0.36 to >50 years (Fig. 2). Lake years when WRT = 1 year provide the best opportunity to evaluate external impacts in a short-term study such as this one.

Percent cut in the DBA of the study lakes varied from 0 to 35% and within 800 m from 0% (SCL800, SPH800) to 35% (SPH20) and was not consistent with the buffer width treatment (Table 1). In these low relief areas, drainage basin boundaries may vary from year to year (Prepas et al. 2001). For example, during wet years, some drainage basins can become joined, whereas in dryer years, they remain distinct.

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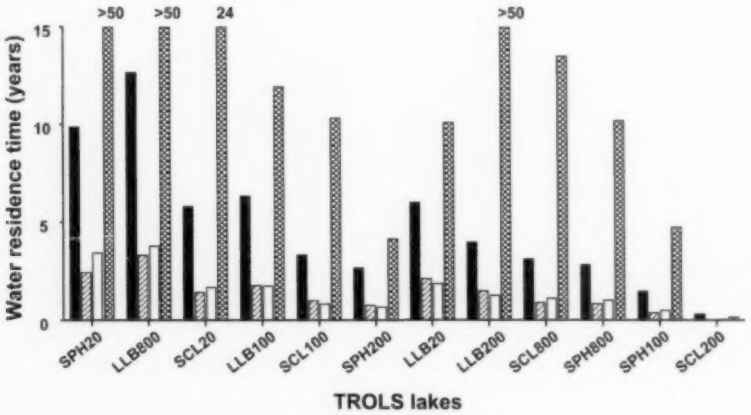
Fig. 2. Annual water residence times for the TROLS lakes for 1995 (solid bars), 1996 (diagonally hatched bars), 1997 (open bars), and 1998 (cross-hatched bars). Water residence times were calculated based on a water year extending from November 1 (previous year) to October 31 (designated year).



Because of this variability, all the study lakes, particularly SCL800 (the only lake with 0% cut in the drainage basin and the lowest watershed slope (1.1%)) likely incurred inputs from nearby harvesting activity during wet years.

Although relatively shallow, the water column of all lakes was at least occasionally thermally stratified during summer. Thermal stratification increased in the posttreatment years, relative to the cooler pretreatments years (illustrated with SPH100; Fig. 3*b*). DO profiles indicated strong chemical stratification in all lakes, with persistent anoxic conditions (DO < 1 $mg \cdot L^{-1}$) over the bottom sediments in the deeper lakes (illustrated with SPH20; Fig. 3*a*). Thermal and chemical stratification was strongest in 1998, a relatively dry and warm year. Following thermal stratification and then mixing, these lakes have a high probability of enhanced surface water P concentrations from internal sources (e.g., Riley and Prepas 1984).

Euphotic zone mean specific conductivity ranged from 107 to 366 $\mu S \cdot cm^{-1}$ in these $CaHCO_2$-dominated lakes (Table 2). pH was consistently high; 4-year means ranged from 7.8 to 9.1. The pH of the riverine SCL200 was an average full unit higher, averaging 9.7, likely from an abundant macrophyte or attached algal population. Overall, the lake water was weakly coloured, with all but one of the headwater lakes having mean colour ≤ 51 mg $Pt \cdot L^{-1}$. LLB200 had coloured water (70 mg $Pt \cdot L^{-1}$) as well as the highest SO_4, NH_4, and DOC concentrations (average 60 $mg \cdot L^{-1}$, 579 $\mu g \cdot L^{-1}$, and 28 $mg \cdot L^{-1}$, respectively) (Table 2). As the highest values were recorded in the dry pretreatment year, they were likely from internal sources.

The average summer values for the nutrient TP (24–132 $\mu g \cdot L^{-1}$) and Secchi depth (1.0–4.2 m) indicate that the lakes range from mesotrophic to hypereutrophic (Table 2). Dissolved IN values were variable; NH_4 made up 78% of DIN. Average 4-year TN to TP ratios varied from 14 to 52 (data not shown). The water chemistry was similar among the three study regions (Table 2) and was similar to that of other lakes in the western sedimentary basin (Mitchell and Prepas 1990).

In the two pretreatment years, TP concentrations in the study lakes were consistent (49.6 ± 5.7 $\mu g \cdot L^{-1}$ in year 1 and 52.8 ± 7.8 $\mu g \cdot L^{-1}$ in year 2 with a mean of 51.2 ± 4.7 $\mu g \cdot L^{-1}$). In the first postharvest year, TP concentrations increased overall ($\chi^2 = 38$, $P < 0.05$) to 73 ± 17 $\mu g \cdot L^{-1}$, whereas in the drier year 4, mean TP dropped to 48 ± 9 $\mu g \cdot L^{-1}$ (Table 3). Particularly in the posttreatment years, within lake categories, TP concentration increased with increased DBA/*V*. Buffer width was not related to changes in pre- and postharvest TP concentration (Fig. 4*a*).

Overall TP concentrations in the stratified lakes were about two thirds of those of the weakly or nonstratified lakes (Table 3). In the pretreatment years, mean summer TP concentration remained fairly consistent overall in the stratified lakes (43.9 ± 7.7 and 42.4 ± 6.8 $\mu g \cdot L^{-1}$), while in the weakly or nonstratified lakes, a small increase in yearly means (56.4 ± 8.2 to 65.3 ± 14.0 $\mu g \cdot L^{-1}$) was not significant ($P \geq 0.05$). Mean TP increased in both lake categories after harvesting, likely due to increased saturation of the organic layer in nearshore areas (Evans et al. 2000), although the increase was only detectable in the shallow lakes ($\chi^2 = 20$, $P < 0.05$). As similar runoff conditions in year 4 (Fig. 1) produced a much reduced response compared with this first posttreatment year (overall change 3 versus 23 $\mu g \cdot L^{-1}$), our results are consistent with a harvest-enhanced wet year response. The posttreatment TP increase in the shallow lakes lasted only 1 year. In contrast, in the stratified lakes, TP concentrations dropped ($\chi^2 = 45$, $P < 0.05$) in the second posttreatment year (Table 3). When TP concentration data from the two pretreatment years were pooled and compared with similar posttreatment data and related to physical features, relative drainage basin area (DBA/*V*) was the strongest correlate ($r^2 = 0.78$, $P << 0.01$).

A concurrent study (Prepas et al. 2001) illustrated that in headwater lakes such as the TROLS lakes, the DBA/*V* or relative catchment area (Table 1) is the physical feature that explained the most variation in lake water TP concentration. In the TROLS lakes, DBA/*V* and WRT are highly correlated ($r^2 = 0.88$, $P << 0.01$). In the wetter first posttreatment year, log-transformed changes in TP concentration were best related to DBA/*V* ($r^2 = 0.56$, $P < 0.01$) and WRT ($r^2 = 0.47$,

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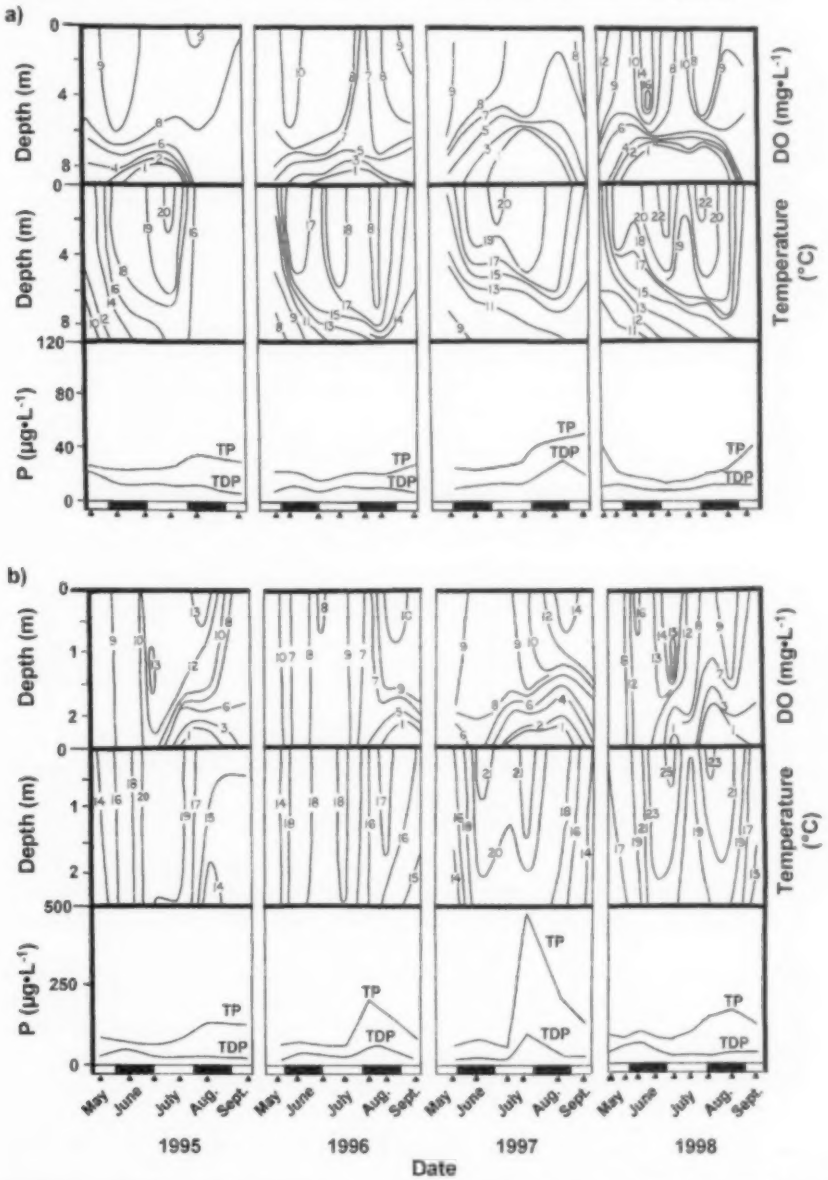
Fig. 3. Thermal and chemical (DO) data organized over time (4-year study) and depth in a (*a*) deep (SPH20) and (*b*) shallow (SPH100) study lake. Euphotic zone TP and TDP concentrations are presented for the same time period.



$P < 0.02$). When percent harvested in the drainage basin was added to the regression as a second independent variable, it contributed a nondetectable 12% to the change in TP concentration ($P > 0.05$). Thus, in the wetter first posttreatment year, the overall increase in TP concentration, although variable, was best related to relative drainage basin area ($\chi^2 = 44$, $P < 0.01$). In the drier second posttreatment year when the shortest lake WRT was 4.1 years (Fig. 2), a small overall decrease in TP was registered relative to pretreatment conditions (Table 3). A large (63–28 µg·L^{-1}) drop in the deeper (i.e., thermally stratified) lakes was offset by a more modest change (85–72 µg·L^{-1}) in the shallower lakes (Table 3).

Although relative watershed size partially explained post-treatment changes in TP, internal factors also played a role. In the pretreatment years, episodic increases in TP concentrations divided themselves roughly into one third internal and two thirds external inputs, and overall, these inputs represented 23% of the average TP concentration (51 µg·L^{-1}) in the lake water. In contrast, although baseline TP remained relatively constant in the posttreatment years (44–35 µg·L^{-1}), external loading represented a larger portion (just over 40%) of the episodic inputs in year 3 compared with 12% in year 4. Internal P loading was most clearly defined in the warmer and drier fourth year of the study (Figs. 1 and 3). For example, TP concentration began increasing in the euphotic zone in the latter half of July 1998 in both the deeper SPH20 and

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Table 2. Water chemistry of the euphotic zone for the TROLS lakes.

Lake	Secchi depth (m)	Colour (mg Pt·L^{-1})	DOC (mg·L^{-1})	Conductivity (μS·cm^{-1})	pH	CO_3 (mg·L^{-1})	HCO_3 (mg·L^{-1})	SO_4 (mg·L^{-1})	Cl (mg·L^{-1})	Ca (mg·L^{-1})	Mg (mg·L^{-1})
SPH20	4.2	20	10.1	213	8.2	0.8	114	1.1	0.4	24	7.9
	3.6–5.1	12–31	10.1–10.4	207–219	8.0–8.4	0–3.4	109–116	1.0–1.2	0.4–0.4	22–26	7.0–9.4
LLB800	2.8	30	12.3	144	8.1	1.9	74	2.5	0.4	16.5	5.7
	1.9–3.2	19–44	10.8–13.4	142–152	7.7–8.7	0–7.5	69–78	1.4–3.5	0.3–0.5	15–19	5.2–7.0
SCL20	1.0	42	16.0	335	8.8	11	167	3.5	0.6	35	18
	0.6–1.5	20–82	12.7–18.5	322–344	8.5–9.0	4.9–22	144–186	3.2–4.1	0.5–0.8	32–38	15–25
LLB100	2.1	39	17.8	107	7.8	0	58	0.3	0.5	9.8	4.7
	1.9–2.6	26–58	16.1–21.7	92–119	7.5–8.2	0–0	51–68	0.1–0.6	0.3–0.7	7.9–12	4.0–5.9
SCL100	1.5	27	12.6	307	8.3	0.1	172	0.3	0.4	34	12
	1.4–1.6	20–38	11.7–13.5	285–336	8.2–8.4	0–0.3	161–189	0.2–0.4	0.2–0.6	32–37	11–15
SPH200	2.8	35	13.7	348	8.1	1.6	186	4.9	1.6	38	15
	2.4–3.5	26–54	12.7–15.5	336–377	7.8–8.3	0–4.4	175–200	2.6–9.4	1.6–1.7	36–42	13–20
LLB20	2.7	37	12.4	255	8.3	2.1	136	5.9	1.0	31	9.4
	1.5–3.3	30–49	11.5–14.4	226–276	7.9–8.7	0–8.5	127–148	4.4–8.2	0.7–1.4	28–35	7.6–13
LLB200	1.7	70	27.7	366	7.9	0	140	60	1.2	48	14
	1.1–2.6	46–100	24.7–33.3	291–417	7.6–8.1	0–0	118–172	45–74	0.9–1.6	42–56	11–20
SCL800	2.0	42	14.6	185	9.1	13	79	0.3	0.3	20	8.6
	1.0–2.7	28–68	12.7–16.5	169–201	8.6–9.5	3.8–19	60–104	0.1–0.5	0.2–0.6	18–23	6.5–11
SPH800	1.7	36	12.9	253	8.5	6.6	127	1.8	0.4	29	9.8
	1.1–2.8	26–58	11.8–13.7	232–277	8.0–9.2	0–17	96–145	1.7–2.1	0.2–0.5	26–32	8.0–13
SPH100	1.1	51	15.3	192	9.0	12	83	0.6	0.4	21	7.3
	0.9–1.4	41–64	13.1–19.7	167–208	8.3–9.4	2.9–18	69–99	0.4–0.9	0.3–0.6	20–22	5.9–9.2
SCL200[a]	1.0	72	19.4	223	9.7	28	62	1.4	0.3	20	10
	0.7–1.3	41–105	17.2–21.9	211–236	9.3–10	19–37	46–78	1.2–1.7	0.1–0.5	14–28	9.0–13

Note: Values are averages and ranges of yearly means (1995–1998) for samples collected between June and August. For dominant cations and anions.
[a]SCL200 is not a headwater lake.

Table 3. Means (±SE) of nutrients, phytoplankton, and zooplankton during preharvest (1995 and 1996) and postharvest years (1997

Lake grouping and year	TP (μg·L^{-1})	DIN (μg·L^{-1})	DOC (mg·m^{-3})	Tchl*a* (μg·L^{-1})	EP (μg·L^{-1})	NEP (μg·L^{-1})	CYA (mg·m^{-3})	NOS (mg·m^{-3})
All (n = 11)								
Preharvest (1995 and 1996)	51.2±4.7	133±82.9	15.2±1.2	16.3±2.2	8.2±1.2	8.1±1.4	3920±874	3035±806
1997	73.0±16.5*	73.8±28.5	15.4±1.3	19.8±3.3	7.3±1.9**	12.5±2.2**	6373±1428*	5155±1316
1998	47.8±9.0*	93.8±38.7	14.4±1.3	21.5±2.7	10.0±1.5**	11.4±1.8**	6992±1172*	5445±1080
Stratified (n = 6)								
Preharvest (1995 and 1996)	43.2±4.9	42.3±11.5	13.8±0.9	16.8±1.8	7.2±0.9	9.6±1.5	5428±1096	4128±985
1997	63.3±17.7	75.1±149.2	14.0±1.3	13.3±2.8	3.5±1.5**	9.8±2.3*	4701±1790	3178±1609
1998	27.5±3.4*	65.1±37.6	13.5±1.0	11.8±2.3	5.1±1.2**	6.7±1.9*	3897±1489	2497±1339
Nonstratified (n = 5)								
Preharvest (1995 and 1996)	60.8±7.8	242±181	16.9±2.3	15.7±4.0	9.4±2.3	6.3±2.4	2110±1320	1725±1244
1997	84.8±31.1*	72.2±29.0	17.0±2.4	27.5±6.1	11.8±3.5	15.7±3.7**	8378±2155**	7528±2031**
1998	72.1±12.4	127.5±74.7	15.6±2.6	32.4±5.0	15.6±2.8	16.8±3.0**	10490±1470**	8778±1640**

Note: Significant differences between pre- and post-harvest values for TP, TChl*a*, EP Chl*a* (<20 μm), NEP Chl*a* (>20 μm), cyanobacteria (CYA), tests. Significant differences between pre- and post-harvest for zooplankton (ZOO) abundance and biomass were analyzed by ANOVAs (*$P < 0.05$; **$P <$

shallower SPH100 lakes (Fig. 3) following a mixing event where accumulated deepwater P was transferred to the euphotic zone (similar to Riley and Prepas 1984). In contrast, 1 year earlier (July 1997), a large increase in euphotic zone TP concentration (>400 μg·L^{-1}; Fig. 3*b*) was registered in the relatively rapidly flushed SPH100 (WRT = 0.5 year in 1997; Fig. 2), associated with the largest rain event that month (45 mm in 24 h) (Fig. 1). During the same storm event, the more slowly flushed SPH20 (WRT = 3.4 years in 1997; see Fig. 2) registered a modest increase (17 μg·L^{-1}) in euphotic zone TP concentration (Fig. 3*a*). Increased TP concentration in year 3 was strongest in those lakes with a relatively short WRT and large DBA/*V* ratio (SPH100, SPH200), while in year 4, increases were greatesr in the shallower and more easily mixed lakes (SCL800, SPH800) (Table 1; Figs. 2 and 4*a*).

Dissolved IN (DIN) concentrations were highly variable (e.g., 20-fold in LLB200; Fig. 4*b*), while the range of variation in mean DOC concentrations was narrow over all lakes and years (9–33 mg·L^{-1}) (Fig. 4*c*; Table 2). The highest DIN and DOC concentrations were recorded in LLB200, which also registered high SO_4 concentrations and colour (Figs. 4*b* and 4*c*). DIN increased in the posttreatment years in SPH200, likely related to removal of a beaver dam and a

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Na ($mg \cdot L^{-1}$)	K ($mg \cdot L^{-1}$)	Si ($\mu g \cdot L^{-1}$)	NO_3 ($\mu g \cdot L^{-1}$)	NH_4 ($\mu g \cdot L^{-1}$)	TP ($\mu g \cdot L^{-1}$)	Chl *a* ($\mu g \cdot L^{-1}$)
2.9	6.1	1580	5.8	12	24	4.0
2.6–3.0	5.7–6.7	1267–1958	2.1–7.2	6.0–21	18–31	2.4–5.0
1.9	4.4	443	10.3	68	48	13
1.4–3.0	4.1–4.7	363–522	4.6–16	16–123	29–58	8.0–19
5.8	4.8	1059	3.8	24	46	19
5.1–6.5	4.5–5.3	351–1485	2.9–4.8	9.8–58	38–50	13–34
2.3	5.6	140	7.4	30	64	17
1.6–3.5	4.7–7.0	76–185	3.1–15	18–51	31–88	7.2–28
7.7	2.7	2790	3.2	4.8	24	11
6.8–8.4	2.3–3.4	1784–3385	2.5–4.1	3.1–5.6	16–28	5.4–23
5.3	2.9	4206	25	138	60	8.8
4.5–6.1	2.7–3.1	1483–5866	3.1–88	18–314	33–135	4.8–14
7.0	2.1	2866	14.5	108	47	11
5.0–8.8	2.0–2.2	2474–3445	5.9–36	61–151	43–50	4.1–20
5.7	5.9	1423	45	579	63	20
4.4–6.9	5.1–7.2	978–2142	9.6–139	71–1722	53–78	4.8–29
4.0	4.0	1517	6.3	18	55	8.8
2.9–4.5	3.1–5.0	830–2620	4.7–9.7	8.7–33	39–78	2.2–20
5.5	2.2	3463	5.0	13	54	15
3.8–6.7	1.7–2.8	2314–4116	3.2–7.9	7.8–23	44–62	6.0–33
5.1	2.0	2362	5.6	57	132	36
4.3–5.9	1.8–2.1	892–3830	3.7–8.3	12–124	88–209	13–61
9.6	2.4	703	4.3	11	58	12
6.0–13	2.0–3.0	416–935	3.3–5.0	5.5–16	42–81	4.4–26

where euphotic zone samples were not available, epilimnion samples were substituted.

and 1998) (SCL200 not included).

CHR ($mg \cdot m^{-3}$)	MIC ($mg \cdot m^{-3}$)	ZOO abundance ($individuals \cdot L^{-1}$)	ZOO biomass ($mg \cdot m^{-3}$)	CLA biomass ($mg \cdot m^{-3}$)	CALA biomass ($mg \cdot m^{-3}$)
170±88	251±101	681±221	69±16	25±9	15±4
520±144*	99±166*	376±193*	27±7*	4±1*	6±2*
533±119*	183±136*	453±162	25±5**	8±2*	4±1*
156±84	359±174	460±173	36±6	17±5	7±2
396±137*	155±284	144±28*	10±2**	2±1*	2±1*
235±114*	27±24	233±24	15±2*	7±3*	4±1*
187±162	120±79	947±437	110±26	34±18	24±7
669±265	31±130**	656±410	47±8	7±2*	9±4*
870±214	359±105**	716±334	36±5*	9±2	5±1*

Nostocales (NOS), Chroococcales (CHR), and *Microcystis* species (MIC) were analyzed by Fisher 0.01). CLA, Cladocera; CALA, Calanoida.

drop in water level of over 1 m in year 3, a condition that persisted through year 4. Episodic internal loading of N from decaying organic matter may explain the predominance of NH_4 as the prevalent form of DIN (Table 2). DOC peaked only in the stratified lakes SCL20 and LLB100 (Fig. 4*c*) and in the nonstratified lake LLB200 having the highest colour (Table 2). DIN and DOC dynamics appear to be unrelated to drainage basin treatments.

Overall, during the first two posttreatment years in this study, evidence was strongest for a treatment TP effect in those lakes and years with a relatively short WRT (≤1 year). The nutrient response of lakes to changing weather conditions was influenced by thermal regime. Other studies of the influence of harvesting on water quality in northern forested areas have focused on relatively deep lakes which are characterized by soft waters (Rask et al. 1998; Carignan et al. 2000; Lamontagne et al. 2000). In these studies of dimictic lakes, TP as well as other leachable nutrient (DOC, DIN) concentrations increased following generally greater watershed disturbance (average 40 and 24%, respectively, compared with 17% in this study). Ours is the first study that we are aware of with relatively shallow lakes where the P re-

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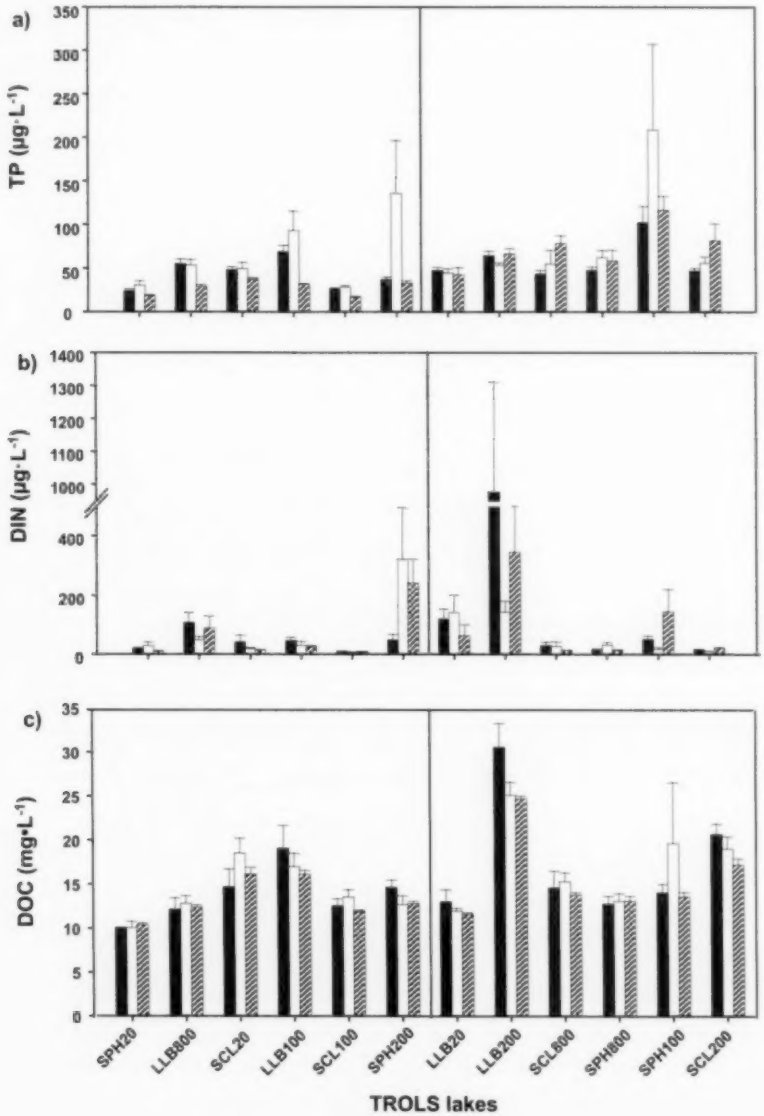
Fig. 4. (*a*) TP, (*b*) DIN, and (*c*) DOC concentrations during preharvest (1995 and 1996 combined (solid bars)) and postharvest years (1997 (open bars) and 1998 (diagonally hatched bars)). Lakes are organized according to thermal stability (the first six are well-stratified lakes and last six are weakly or nonstratified lakes) and in each subset arranged in order of decreasing water residence times. Bars represent means (±SE) for euphotic zone samples collected between June and August of each year.



sponse following watershed harvest was generally magnified where the water column was weakly or nonstratified and DIN and DOC concentrations did not respond detectably.

Phytoplankton community responses to harvesting

Prior to harvesting, mean TChl*a* for all lakes was 16 µg·L^{-1}, composed equally of EP and NEP phytoplankton (8 µg·L^{-1}) (Table 3). Stratified and nonstratified lakes supported similar mean TChl*a*, 16.8 and 15.7 µg·L^{-1}, respectively. However, EP Chl *a* represented 60% of TChl*a* in the nonstratified lakes and only 42% in the stratified lakes. After harvest, overall TChl*a* was 20 and 22 µg·L^{-1} in year 3 and year 4, respectively, for all lakes. Postharvest TChl*a*, EP Chl *a*, and NEP Chl *a* were lower in the stratified than in the nonstratified lakes (Table 3; Fig. 5*a*), and EP Chl *a* represented only 40% of TChl*a* in all lakes overall.

Cyanobacteria contributed 43 and 67% of phytoplankton biomass overall pre- and postharvest, respectively (Fig. 5*b*). In lakes where cyanobacteria were scarce or absent (LLB100 overall, LLB200 and SPH800 in preharvest years), the more abundant taxa were the flagellates (mainly Cryptophyta and dinoflagellates) (Fig. 5*b*). In lakes where cyanobacteria were

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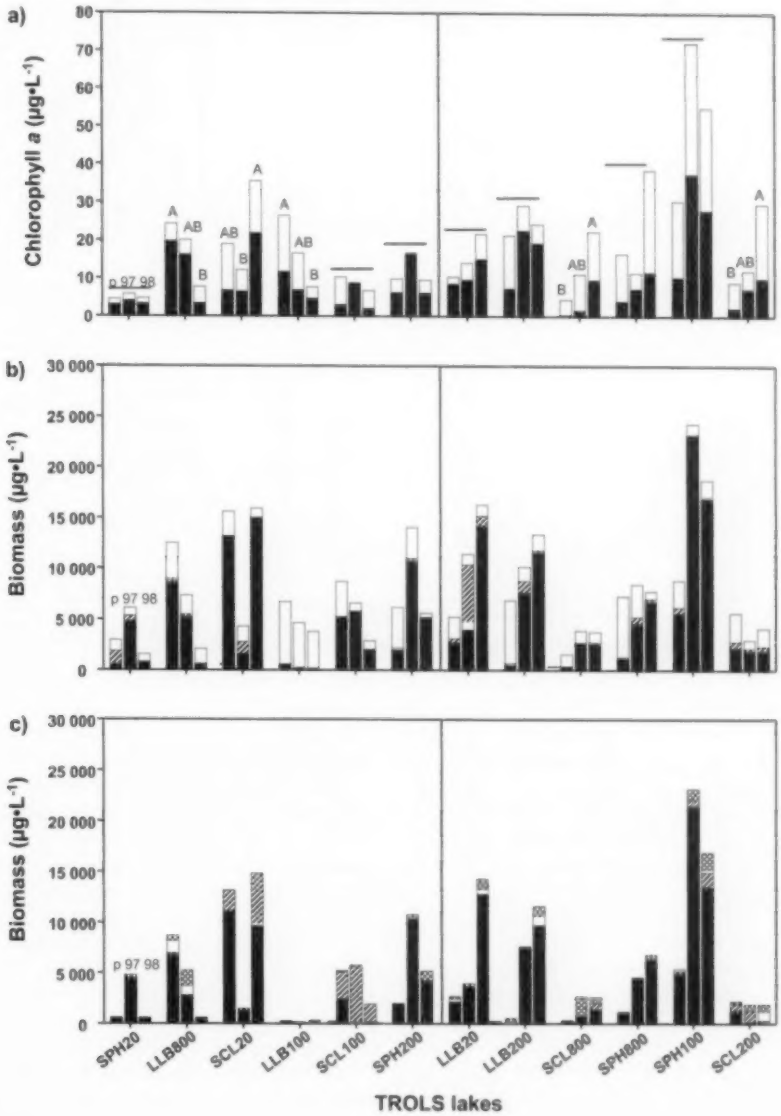
Fig. 5. (*a*) Lake total Chl *a* concentration separated into edible (<20 µm (open bars)) and nonedible fractions (>20 µm (solid bars)) during preharvest (1995 and 1996 combined (p)) and postharvest years (1997 (97) and 1998 (98)). Lakes are organized as in Fig. 4. Capital letters indicate a significant difference ($P < 0.05$). (*b*) Biomass of the main phytoplankton taxa for the TROLS lakes. Solid bars, blue-greens; diagonally hatched bars, flagellates; open bars, diatoms. Organized as in Fig. 5*a*. (*c*) Biomass of cyanobacterial functional taxa. Solid bars, *Aphanizomenon–Anabaena* association; open bars, Oscillatoriaceae; diagonally hatched bars, *Microcystis*; cross-hatched bars, chroococcales other than *Microcystis*. Organized as in Fig. 5*a*.



present, heterocystous nitrogen fixers (N_2 fixers) (Nostocales) were the most abundant, with *Aphanizomenon–Anabaena* being the most common species association (SCL20, LLB20, SHP200, SPH100) and *Gloeothrichia echinulata* being the dominant species in LLB800 (Fig. 5*c*). Within the cyanobacteria-dominated lakes, Oscillatoriales followed the N_2 fixers in importance; however, in SCL100, Oscillatoriales were the dominant algae (Fig. 5*c*).

Although NEP Chl *a* increased overall after harvesting, phytoplankton response in the posttreatment years varied depending on lake thermal structure. In stratified lakes, EP and NEP Chl *a* decreased after harvesting ($P < 0.05$), whereas in nonstratified lakes, NEP Chl *a* increased ($P < 0.01$) (Table 3). The increase in NEP Chl *a* in weakly or nonstratified lakes corresponded to an increase in the biomass of total cyanobacteria, mainly Nostocales and *Microcystis* (only during year 4). Furthermore, MCLR, the most common cyanotoxin in prairie lakes, increased 10-fold in year 3 relative to

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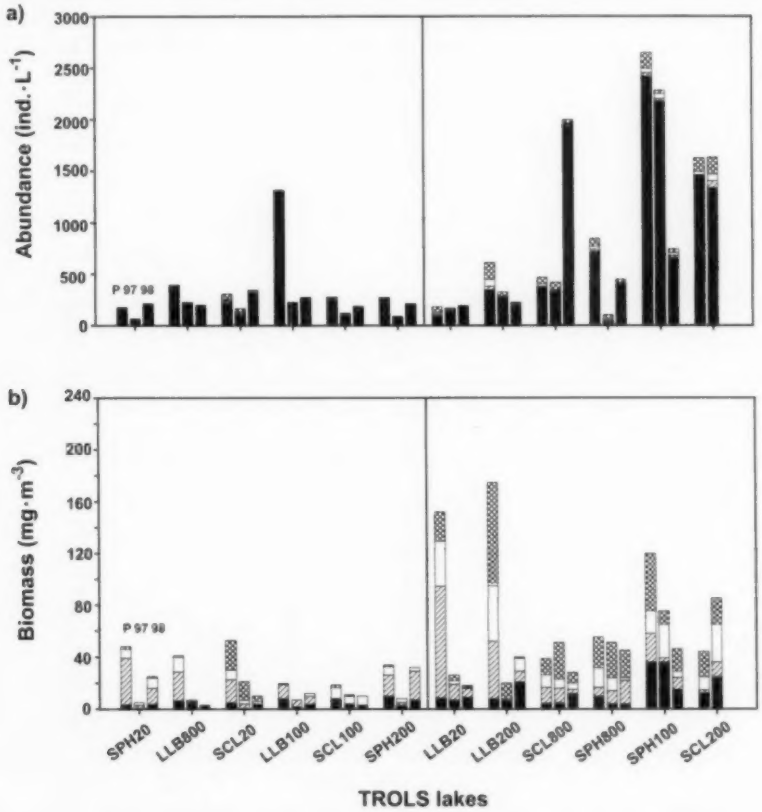
Fig. 6. Zooplankton (*a*) abundance and (*b*) biomass in the TROLS lakes. Organized as in Figs. 4 and 5. Solid bars, Rotifera; diagonally hatched bars, Cladocera; open bars, Calanoida; cross-hatched bars, Cyclopoida.



pretreatment ($t = 3.7$, $P < 0.01$) and remained high in year 4 (2.9 ± 1.4, 23 ± 5.3, and 37 ± 23 μg MCLR·g^{-1}, respectively). The MCLR increase was primarily observed in the nonstratified lakes ($t = 3.9$, $P < 0.02$). Relationships between MCLR and phytoplankton were particularly strong with *Aphanizomenon gracile* ($r^2 = 0.58$ and 0.51, $P < 0.05$ and < 0.02) and *Limnothrotix redeckii* ($r^2 = 0.45$ and 0.43, $P < 0.03$, in the stratified and nonstratified lakes, respectively). Cyanobacteria and associated toxins have been identified previously south of our study lakes in regions with watershed disturbance (Kotak et al. 1995). In our study, pretreatment MCLR concentrations were low, while both cyanobacterial biomass and the predominant cyanotoxin MCLR increased in the postharvest period.

Increased cyanobacterial dominance is expected with enhanced P content of lake water and has been reported elsewhere both in sedimentary and in hardrock basins (Rask et al. 1998). However, no other study has monitored a cyanotoxin before and after watershed disturbance or within the context of contrasting mixing regimes. Given the variability within cyanobacterial and cyanotoxin communities (Kotak et al. 1995) and the dynamic biogeochemistry of these lakes, an in-depth monitoring and expanded treatment program will be required to comprehend phytoplankton responses to watershed disturbance.

Zooplankton community responses to harvesting

Prior to harvesting, total zooplankton mean abundance ranged from a minimum of 170 individuals·L^{-1} (SPH20) to a maximum of 2643 individuals·L^{-1} (SPH100), with a mean density of 681 individuals·L^{-1} (Fig. 6*a*; Table 3). Total zooplankton mean biomass varied from 18 mg·m^{-3} (SCL100) to 175 mg·m^{-3} (LLB200), with a mean of 69 mg·m^{-3} (Fig. 6*b*; Table 3). Zooplankton abundance generally increased from lakes with a smaller drainage ratio (SPH20) to lakes with a larger drainage ratio (SPH100) (Fig. 6*a*; Table 1). Some lakes (LLB20, LLB200, SPH100) had high zooplankton biomass prior to harvesting.

After harvest, zooplankton abundance ranged from 62 individuals·L^{-1} (SPH20) to 2280 individuals·L^{-1} (SPH100), with a mean of 376 individuals·L^{-1} in 1997. In 1998, the range of zooplankton abundance narrowed from 183 individuals·L^{-1} (SCL100) to 1994 individuals·L^{-1} (SCL800), with a mean of 453 individuals·L^{-1} (Fig. 6*a*; Table 3). Zooplankton biomass varied from 5 mg·m^{-3} (SPH20) to 75 mg·m^{-3} (SPH100) in 1997 and from 3 mg·m^{-3} (LLB800) to 46 mg·m^{-3} (SPH100) in 1998 (Fig. 6*b*). Mean biomass was 27 and 25 mg·m^{-3} in 1997 and 1998, respectively (Table 3).

Over the 4-year study, abundance and biomass of zooplankton were higher in weakly or nonstratified lakes than in stratified lakes ($P < 0.05$) (Fig. 6; Table 3). When consider-

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Table 4. Mixed stepwise regressions between TChl*a*, EP Chl *a*, NEP Chl *a*, total (CYA), N_2-fixer, and nonfixer cyanobacterial fractions and lake morphometry, physicochemical, and zooplankton variables (only variables with $P < 0.05$ are retained and presented with partial r^2).

Dependent variable	Regression model	n	r^2_{adj}	P
All lakes				
log TChl*a*	−0.67 + 0.97 log TP − 0.12 Secchi + 0.55 log Z_{max} (0.61) (0.16) (0.08)	44	0.83	<0.0001
log EP Chl *a*	−0.14 + 0.49 log ZOO_{dens} − 0.16 Secchi (0.54) (0.15)	44	0.67	<0.0001
log NEP Chl *a*	−2.16 + 1.33 log TP + 1.01 log Z_{max} (0.28) (0.18)	44	0.44	<0.0001
log CYA	10.03 − 0.58 Secchi − 1.99 log TDN + 0.98 log ZOO_{biom} − 1.07 log CYC_{dens} (0.25) (0.17) (0.12) (0.11)	33	0.59	<0.0001
log N_2 fixers	11.36 + 0.59 Shade EZ − 3.95 log TDN − 5.66 log Z_{epi} + 1.30 log TN (0.18) (0.17) (0.13) (0.07)	33	0.48	0.0002
log nonfixers	7.15 − 0.38 Secchi − 1.38 log TDN (0.25) (0.19)	33	0.45	0.0002
Weakly or nonstratified lakes				
log TChl*a*	−1.85 + 1.32 log TP + 1.59 log Z_{max} − 0.19 Secchi (0.68) (0.25) (0.17)	20	0.97	<0.0001
log EP Chl *a*	−0.04 − 0.24 Secchi + 0.76 log TP (0.74) (0.11)	20	0.83	<0.0001
log NEP Chl *a*	−5.58 + 3.36 log Z_{max} + 2.40 log TP (0.46) (0.37)	20	0.80	<0.0001
log CYA	−5.96 + 4.58 log TP − 1.39 log CAL_{dens} + 2.78 log COND − 3.17 log DOC (0.34) (0.33) (0.10) (0.06)	20	0.77	<0.0001
log N_2 fixers	2.99 − 1.62 log CYC_{dens} + 3.89 log TP − 1.62 log DOC (0.30) (0.25) (0.18)	20	0.65	<0.0001
log nonfixers	−1.07 + 1.89 log TP	20	0.46	0.0006
Well-stratified lakes				
log TChl*a*	0.12 + 0.78 log TP − 0.11 Secchi (0.60) (0.24)	24	0.83	<0.0001
log EP Chl *a*	−2.25 + 0.81 logZOO_{dens} − 0.11 Secchi + 1.33 log Z_{max} (0.44) (0.16) (0.09)	24	0.64	<0.0001
log NEP Chl *a*	−0.52 + 0.88 log TP	24	0.34	0.0016
log CYA	7.11 − 0.57 Secchi − 2.17 log TP + 0.28 Shade EZ − 0.60 log CYC_{dens} (0.28) (0.23) (0.15) (0.09)	24	0.71	<0.0001
log N_2 fixers	−3.19 + 0.28 Shade EZ − 1.64 log CYC_{dens} + 3.07 log ALK − 0.37 Secchi (0.28) (0.19) (0.19) (0.13)	24	0.75	<0.0001
log nonfixers	2.18 + 1.27 log CAL_{dens} − 1.02 log CLA_{biom} (0.35) (0.31)	24	0.63	<0.0001

Note: TDN and TN concentrations not available for 1998. ALK, alkalinity; COND, conductivity; Shade EZ, algal self-shading index; Secchi, Secchi disc depth; Z_{epi}, epilimnion depth; CAL_{dens}, Calanoida abundance; CYC_{dens}, Cyclopoida abundance; CLA_{biom}, Cladocera biomass; ZOO_{biom}, total zooplankton biomass.

ing zooplankton groups, copepod abundance and biomass, cladoceran abundance, and rotifer biomass were higher in the former than in the latter ($P < 0.01$) (data not presented).

Overall, zooplankton community structure in the 12 TROLS lakes was similar to that reported in eutrophic and shallow lakes in the Boreal Plain (Pinel-Alloul 1993; Ghadouani et al. 1998). Rotifers were the most abundant in all lakes (Fig. 6*a*), whereas cladocerans and calanoid or cyclopoid copepods contributed the most to biomass (Fig. 6*b*). Among the rotifers, *Keratella cochlearis*, *Conochilus* sp., *Polyarthra vulgaris*, or *Pompholix* sp. were the most abundant. In terms of biomass, the cladocerans *Daphnia pulicaria* and *Daphnia pulex*, the calanoid *Skistodiaptomus oregonensis*, and the cyclopoid *Diacyclops bicuspidatus thomasi* were dominant.

Zooplankton biomass decreased postharvest in all lakes pooled, in the stratified (years 3 and 4) and nonstratified lakes (year 4) ($P < 0.05$) (Table 3). When all lakes were pooled, calanoid and cladoceran abundance and biomass were lower postharvest ($P < 0.05$). In the stratified lakes, rotifers (year 3, data not shown), cladocerans, and calanoids (years 3 and 4) declined postharvest ($P < 0.05$) (Table 3). In weakly or nonstratified lakes, cyclopoids and calanoids decreased after harvesting ($P < 0.05$) (Table 3). Overall, rotifers showed weak pre- to post-harvest changes in biomass compared with crustaceans. Except for the 800-m-buffered lakes (SPH800, SCL800), the relative contribution of rotifers to total biomass tended to increase during postharvest years, whereas the biomass of cladocerans and copepods tended to decline. Although there were detectable pre- to post-harvest differences, biomass and abundance of total

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zooplankton and groups were indistinguishable in the two postharvest years ($P > 0.05$).

Similar to our findings, (*i*) when 15–33% of the watershed was harvested in a Finnish study, zooplankton species assemblages and rotifer abundance did not change and (*ii*) when an average of 38% of the watershed was harvested on the Precambrian Shield, calanoid biomass decreased. In contrast with our results, a slight increase in crustacean abundance was noted in shallow Finnish lakes, while no detectable variation occurred in total zooplankton and cladoceran biomass after harvesting on the Precambrian Shield (Rask et al. 1998; Patoine et al. 2000). Although our study indicated decreased zooplankton abundance and biomass after harvest, we cannot statistically evaluate the pre- and postharvest differences in relation to buffer strip width: (*i*) the use of one composite zooplankton sample per lake (no replication) precluded variance testing between years and lakes; however, there is no systematic trend in zooplankton density or biomass related to buffer strip width (Fig. 6); (*ii*) some control lakes (SCL800, SPH 800) with low harvesting in their watershed (0–5% of the watersheds) had as much year-to-year variation in zooplankton abundance and biomass as lakes with more of their watershed harvested. The difficulty in linking changes in zooplankton to harvesting practices could be confounded by year-to-year changes in climate. We thought that zooplankton abundance would be higher during the dry years, 1995 and 1998. However, a detailed analysis (data not shown) indicated that although zooplankton abundance was almost always higher during the dry year (1995) than during the wetter year (1996), in 1998, another dry year, higher abundance of zooplankton was noted only in half of the lakes. Furthermore, when we compared the two wet years (1996 and 1997), zooplankton abundance was lower only in 1997. Thus, the drop in zooplankton abundance in the posttreatment years is likely related to treatment and not to climate.

Relationships with watershed and lake conditions

Variance in Chl *a* biomass and phytoplankton functional groups in the TROLS lakes was mainly related to nutrients, lake depth, underwater light climate, and zooplankton community (Table 4). Relationships with environmental factors varied with lake thermal structure. In all lakes, TP concentration was a good predictor of TChl*a* and NEP Chl *a*, independent of thermal characteristics, as is common in temperate lakes (Planas et al. 2000). However, TP was related to EP Chl *a* only in weakly or nonstratified lakes. Increases in TChl*a* in relation to TP were greater in weakly or nonstratified lakes than in stratified lakes (partial r^2 of 1.32 versus 0.78, $P < 0.001$) (Table 4). TP was also a good predictor of the biomass of total cyanobacteria and of N_2 fixers or nonfixers, only in the weakly or nonstratified lakes. In stratified lakes, light-related variables and (or) zooplankton density or biomass were important correlates of N_2-fixing or nonfixing cyanobacteria. The strong relationship between N_2-fixing cyanobacteria and TP in polymictic lakes has been reported previously by Trimbee and Prepas (1987), who suggested that the correlation between heterocystous N_2 fixers and TN to TP ratios could be spurious, stemming from an increase in P rather than a decrease in the ratio. It could be that the relationship between cyanobacterial functional groups and P in polymictic lakes of this study is also spurious. Phosphorus has been reported as the main nutrient that contributes to the increase in akinete germination of some *Anabaena* species (Van Dok and Har 1997). Akinete germination and filamentous growth of N_2 fixers such as *Anabaena*, *Aphanizomenon*, and *Gloetrichia* (another dominant heterocystous cyanobacterium in some of the TROLS lakes) occur at the bottom of lakes if light is available. The filaments of these species migrate through the water column of polymictic lakes (Jacobsen 1994). This life cycle, which incorporates germination and growth on the lake bottom and migration in the water column, can explain the high biomass of heterocystic N_2 fixers in our weakly or nonstratified lakes where light reaches the bottom. Furthermore, migration of these species into the water column can contribute to internal P loading (Jacobsen 1994). Thus, correlation between N_2 fixers and TP, as observed in weakly or nonstratified lakes, could also be spurious, stemming from a higher TP concentration per unit biomass in these filamentous bottom growers. When all lakes were considered together, total cyanobacterial and the N_2-fixer and nonfixer functional groups were negatively related to increased TDN concentration (Table 4).

Light appears to impair phytoplankton growth in these lakes, as evidenced by the relationship between phytoplankton variables (TChl*a*, EP, cyanobacteria, N_2 fixers, nonfixers) and either Secchi or euphotic depth (negative) or autoshading index (positive) in almost all multiple regressions (Table 4). Low light can limit the growth of some taxa, while for others, it can promote dominance. The negative relationships between cyanobacteria and water transparency may be a consequence of algal shading rather than a cause. Thus, cyanobacteria could both be favoured in shady conditions and promote autoshading, as suggested by the positive relationship between cyanobacterial N_2 fixers and algal self-shading index in all lakes pooled as well as in the stratified lakes (Table 4). In this study, although N_2-fixing cyanobacteria were the most abundant (Table 3), Oscillatoriales were the most abundant nonfixing cyanobacteria, particularly in lakes that were thermally stable. The negative relationship between the biomass of nonfixers and Secchi depth may be related to the positive relationship between Oscillatoriales and turbidity (SCL100, SCL20) (Table 2). Moreover, it is known that Oscillatoriales cause higher turbidity per unit of P than other algae contributing to self-shading (Scheffer et al. 1997).

Zooplankton–phytoplankton trophodynamics were characterized by strong positive relationships between EP Chl *a* and zooplankton abundance in all lakes pooled and in the stratified lakes (Table 4). This is mainly due to strong correlations between EP Chl *a* and rotifer abundance ($r^2 > 0.6$), which represented most of the zooplankton (Fig. 6*a*). Although the biomasses of zooplankton and cyanobacteria were positively related across all lakes (Table 4; Figs. 5*b* and 6*b*), negative relationships were noted between total cyanobacterial or N_2-fixer and nonfixer functional groups and the abundance of cyclopoid and calanoid copepods and the biomass of cladocerans (Table 4). We found the highest abundance and biomass of these crustaceans in LLB20 and LLB200 in preharvest years (Fig. 6) associated with low biomass of cyanobacteria (Fig. 5*b*). A previous study showed that the biomass of large cladocerans like *D. pulicaria* in

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nearby eutrophic lakes varied inversely with filamentous and colonial cyanobacteria (Ghadouani et al. 1998).

As zooplankton communities may also be influenced by top-down cascades (Carpenter and Kitchell 1993), we examined the relationships between zooplankton variables and vertebrate (planktivore CPUE) and invertebrate predators (*Chaoborus* larvae abundance) and found only limited patterns. In stratified lakes, the abundance and biomass of copepods and the abundance of fish planktivores were positively related (r^2 = 0.7–0.8, $P < 0.05$). In the deepest lakes, crustaceans may find a refuge in hypolimnetic waters or migrate diurnally to avoid fish predators, in contrast with shallow lakes where predator avoidance may be more difficult.

Watershed and lake morphometry influenced lake TP and Chl *a* concentrations. Posttreatment changes in TP concentrations were positively related to the relative size of the watershed (r^2 = 0.4–0.6). TChl*a* and NEP Chl *a* were negatively related to WRT (r^2 = 0.3, $P < 0.01$), consistent with higher Chl *a* biomass in poorly stratified lakes. Abundance of total zooplankton, rotifers, and calanoid and cyclopoid copepods increased in lakes with higher drainage ratios ($r^2 > 0.5$), as observed in Fig. 6*a*. Similar relationships were observed when considering zooplankton biomass but with low correlation coefficients (r^2 = 0.3–0.5). The copepods were less abundant in lakes with longer WRT (r^2 = –0.4 to –0.6). These planktonic relationships with lake and watershed characteristics were generally stronger in weakly or nonstratified lakes.

In conclusion, although the effects of harvesting were not related to buffer strip width, we found that even a modest disturbance in the watershed can enhance euphotic zone TP under specific climatic and watershed or lake conditions. In this 2-year posttreatment study, lakes with the relatively largest drainage basin, and thus shorter residence time, had the strongest response, which was both magnified and extended in the weakly or non-thermally-stratified lakes. Changes in the nutrients status of the lakes were reflected immediately in the phytoplankton and associated toxin communities. In contrast, the drop in zooplankton biomass and abundance after harvesting was most pronounced in the stratified lakes, where it was related to higher biomass of cyanobacteria and their toxins. We also found stronger positive relationships between nutrients and chlorophyll biomass and macrozooplankton abundance and biomass in the stratified lakes, whereas rotifer responses to environmental factors were stronger in weakly or nonstratified lakes. We predict that in these slowly flushed systems, where P cycling from bottom sediments is the norm, the response may continue for a decade or more and be positively related to runoff.

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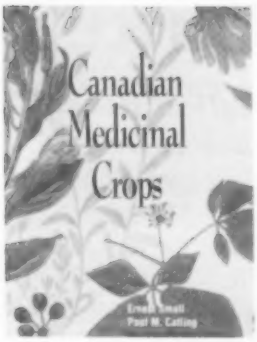

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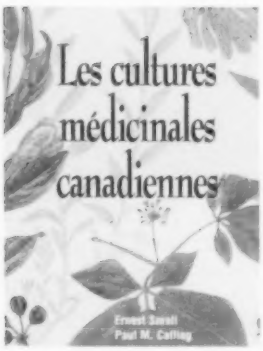

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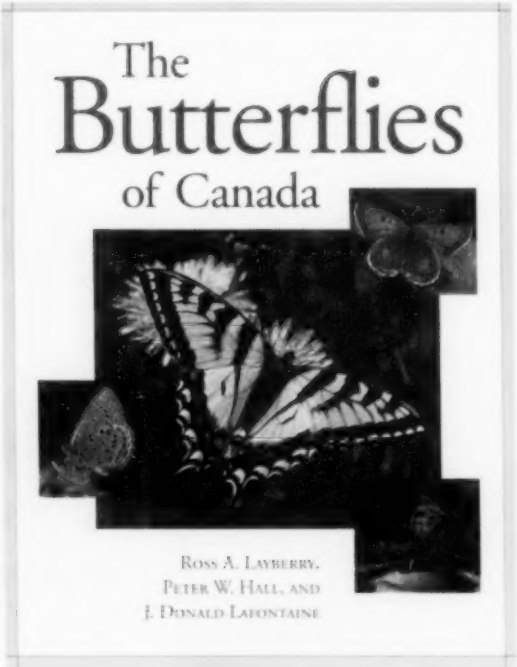

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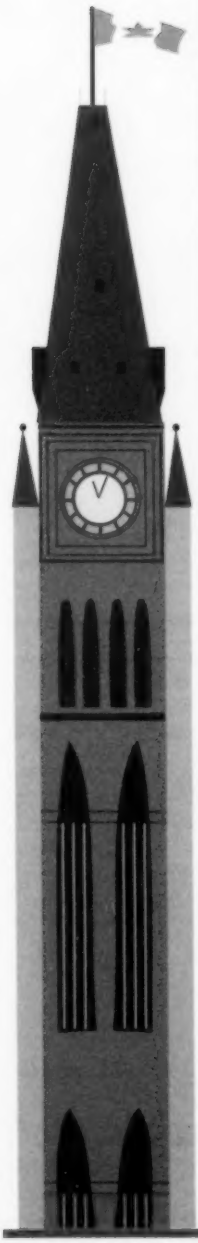

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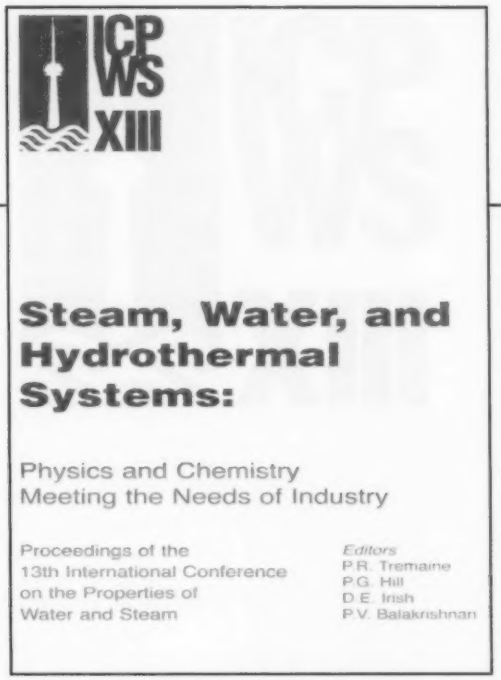

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